Anti-oxidative Effects of Rooibos Tea Extract on Autoxidation and Thermal Oxidation of Lipids

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Abstract: Powdered rooibos tea extract (RTE), which is rich in polyphenols, is made from rooibos tea by freeze-drying. “Rooibos” is Afrikaans for “red bush,” and the scientific name is “Aspalathus linearis.” It is a broom-like member of the legume family of plants and is used to make an herbal tea which has been popular in South Africa for generations and is now consumed in many countries. In the present work, the anti-oxidative effect of RTE on oils and fats in autoxidation or thermal oxidation was studied, and it was confirmed that RTE has a very strong anti-oxidative effect on emulsifying oils owing to the water-soluble polyphenols such as rutin and quercetin contained in RTE. RTE was found to have a strong ability to quench radicals generated in the water phase, and to confer higher thermal stability against deep fat frying than tocopherol. But RTE showed little anti-oxidative effect on frying oil because of its lower oil-solubility.

Key words: Rooibos tea extract (RTE), anti-oxidative activity, rutin, quercetin, emulsifying oil, deep fat frying, autoxidation, thermal oxidation

1 INTRODUCTION

Although tocopherol (Toc) is used at present as the main natural anti-oxidant for the prevention of lipid oxidation, some plant polyphenols have been drawing much attention as novel alternatives in recent years. Green tea is a typical drink rich in catechins, and first-harvest tea leaves contain about 12-14% catechins, whereas second-harvest ones contain about 14-15%. The tea leaves also contain polysaccharides, some pigments such as nitrogen-containing compounds such as caffeine and theanine, various vitamins, flavonols, chlorophyll, anthocyanin and carotenoids, and trace metals, all of which have different physiological properties. Catechins are known to prevent the oxidation of oils and fats, and to scavenge active oxygen and radicals.

Epigallocatechin gallate (EGCG) is the main catechin contained in green-tea leaves, accounting for about 60% of all catechins therein. It has been reported that the anti-oxidative and radical-scavenging activities of tea catechins derive mainly from EGCG. Tsuda has demonstrated in an in vitro test that the anthocyanin in tea leaves has anti-oxidative activity, active-oxygen-scavenging activity, suppression activity for UV injury, and tyrosinase inhibition activity, and inhibits the nitration of tyrosine residues in human LDL by peroxy nitric acid; he has also found correlations between those activities and the chemical structure of anthocyanin.

Recently, rooibos (Afrikaans for “red bush”; scientific name, Aspalathus linearis) tea, which is known as a popular herbal tea in South Africa and contains polyphenols classified into flavonoids, has been drawing much attention. Rooibos tea is purported to relieve nervous tension, allergies and digestive problems. There are many reports on traditional medicinal uses of rooibos tea in South Africa include alleviating infantile colic, allergies, asthma and dermatological problems.

The flavonoids contained in rooibos tea are estimated to be the glycosides luteolin and quercetin, and these compounds are considered to eliminate active oxygen. In fact, Ohta has pioneered the study of the anti-oxidative effect of quercetin on cotton seed oil, as reported by Green Bank. The authors have also reported on the anti-oxidative effects of grape seed extracts and powdered rooibos tea extract (RTE) made by freeze-drying; these substances were examined by autoxidation tests with high linoleic safflower fatty-acid methyl ester in non-aqueous and emulsifying systems. As for the effects of those natural prod-
ucts, grape seed extracts in a non-aqueous system and RTE in an emulsifying system showed higher effects than Toc. However, if different synergists could be added to those natural products, much stronger effects would be obtained. Therefore, the authors have studied the synergistic effects of phosphatidyl ethanolamine (PE), ascorbic acid (AA) and citric acid (CA) on RTE, and have found that PE showed an excellent synergistic effect

In that study, the necessity to perform further experiments became apparent because there were some differences in the effects depending on the added amounts of synergist by weight and molarity. In addition, the effects of those products on the thermal oxidation of deep-frying oil remained unelucidated.

In the present study, we turned our attention again to RTE as an alternative for Toc, which is a widely used natural anti-oxidant, and reconfirmed its anti-oxidative activity in autoxidation and evaluated that in thermal oxidation by comparison with that of Toc. In addition, the determination of the concentration and the identification of the active components in RTE were also carried out.

2 EXPERIMENTS
2.1 Samples and reagents

The high linoleic safflower oil fatty-acid methyl ester used as a substrate in the autoxidation test was prepared as the previous study. The purified soybean oil used as a substrate in the thermal oxidation test was prepared by the silica-gel column chromatographic fractionation of soybean oil donated from Showa Sangyo Co., Ltd. (Funabashi, Japan) with hexane/diethyl ether (85/15 v/v) as the elution solvent to eliminate the lipid peroxides and Toc. All phenolic compounds and synergists such as the RTE, the prooxidants, the lipids (PL). The separation of the PL fraction was carried out with ethanol in the food and cosmetics industries, although the extraction of polyphenols is generally carried out with ethanol in the food and cosmetics industries,

2.2 Characterization of samples
2.2.1 Lipid composition

The lipid composition was analyzed by TLC-FID on a Iatroscan, model MK-5 (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan). The analysis was carried out with benzene/chloroform/acetic acid (35/15/1 v/v/v) as the first developing solvent, which is capable of separating the simple lipids (TAG, DAG, MAG and FA) from the phospholipids (PL). The separation of the PL fraction was carried out with chloroform/methanol/water (75/25/2 v/v/v), as necessary.

2.2.2 Fatty-acid composition and residual ratio of methyl linoleate

The fatty-acid compositions were analyzed on a Shimadzu gas chromatograph, model GC-18 (Shimadzu Corporation, Kyoto, Japan), equipped with a 0.25 mm × 25 m capillary column (Shinwa Chemical Industries, Ltd., Kyoto, Japan) at 200 °C and FID. The residual ratio of methyl linoleate was calculated as the ratio against the methyl palmitate contained in the sample lipids regarded as an internal standard; that is, the residual methyl linoleate ratio was calculated in the time course as 100% of the content at the starting time of autoxidation. Prior to the analysis of the fatty-acid composition of PE, the constituted fatty acids were methyl-esterified by the Jham method.

2.2.3 Determination of Toc

The residual amounts of Toc contained in the high linoleic safflower oil fatty-acid methyl ester and soybean oil used as substrates in the autoxidation and thermal oxidation tests were determined on a JASCO HPLC, model BIP-1, equipped with a Fine SIL-5 column (4.6×250 mm, particle size: 5 μm) and a fluorescent detector, model FP-2020, (Ex: 295 nm, Em: 325 nm) using hexane/2-propanol (124/1 v/v) as a mobile phase with a flow rate of 0.7 mL/min.

2.2.4 Tracing of the oxidation behavior

The time courses of autoxidized lipids to reconfirm the previous report and thermal oxidized lipids were traced by the measuring the peroxide value (PV), conjugated diene content, anisidine value (AnV) and acid value (AV), as described in the “Standard Method for the Analysis of Fats, Oils and Related Materials” edited by the Japan Oil Chemists’ Society. The polar compound content was measured by the method reported by Hara, et al. The polymer content was determined on a JASCO gel permeation chromatograph (GPC), model PU-2080-Plus, equipped with a KF-802.5-C-508007 column (8.0×300 mm) and an RI-2031-Plus detector, by running tetrahydrofuran at a flow rate of 0.7 mL/min.

2.3 Separation of the polyphenols contained in RTE
2.3.1 Extraction with alcohol

Although the extraction of polyphenols is generally carried out with ethanol in the food and cosmetics industries,
methanol was used in the present experiment because of its high solubility for RTE.

2.3.2 Silica-gel column chromatographic fractionation
The polyphenols contained in RTE were fractionated by silica-gel column chromatography.

Heat-activated column chromatographic silica gel 60 (Merck & Co., Inc., USA) was poured into a 25 × 400-mm glass column, and 1 g of RTE was loaded and fractionated with methanol/ethanol/diethyl ether (70/10/20 v/v/v) as the elution solvent.

2.3.3 Identification of the compounds in the RTE concentrate by TLC analysis
To determine the chemical structure of the compounds in the RTE concentrate described in 2.3.2, 1% RTE concentrate-methanol solution was loaded on a TLC fluorescent silica-gel 60F254 plate and then developed with methanol/ethanol/diethyl ether (70/10/20 v/v/v). The separated spots were visualized under a UV lamp.

2.3.4 Identification of the compounds in the RTE concentrate by FT-IR analysis
To determine the chemical structure of the compounds contained in the RTE concentrate described in 2.3.2, FT-IR analysis was also performed by the KBr tablet method on a JASCO FT-IR, model 5300.

2.3.5 Identification of the compounds in the RTE concentrate by 1H-NMR analysis
To confirm the chemical structure of the compounds contained in the RTE concentrate described in 2.3.2, 1H-NMR analysis (δ, 400 MHz, CD3OD) was performed on a JEOL JIM-LA400D NMR spectrometer (JEOL, Ltd., Tokyo, Japan). The analysis was carried out using a RTE-heavy methanol solution and tetramethylsilane as an internal standard.

2.4 Determination of the polyphenol compounds
The polyphenols contained in RTE, rutin, quercetin, the RTE concentrate, and Pro were determined by colorimetry with Folin-Ciocalteu’s reagent: 1,250 ppm for RTE, 1,000 ppm for rutin, 550 ppm for quercetin, 500 ppm for the RTE concentrate, and 500 ppm for Toc. In addition, a sample to which 500 ppm of Toc and 1,250 ppm of RTE were added was also examined to evaluate synergistic effect of RTE on Toc. To evaluate the anti-oxidative effect of the polyphenols tested within a brief period, 1,000 ppm of AIBN, which is an oil-soluble radical generator that acts as an oxidation promotor, was added to the substrate.

2.6.2 Autoxidation in an emulsifying system
Autoxidized substrates were prepared by adjusting the polyphenol contents in the same way as in the non-aqueous autoxidation test described in 2.6.1. Five hundred ppm of each polyphenol were added to the substrates characterized in Table 1, and then the substrates were emulsified to O/W=37:63 (v/v), to which 1,000 ppm of oil-soluble AIBN or water-soluble AAPD as the radical generator was added to compare the radical-scavenging ability of RTE in the two cases39). Autoxidation was carried out in an incubator (Iwashita Co., Ltd, Tokyo, Japan) under constant shaking at 1,000 rpm and 30°C.

2.6.3 Examination of the optimum addition amounts of three synergists in a non-aqueous system
Soybean PE (PV: 6.8 me/kg) at a purity of 99.2%, AP at a purity of 99.5%, and CA at a purity of 99.5% were used as anti-oxidative synergists for RTE, and their optimum addition amounts were examined in a non-aqueous autoxidation test. The high linoleic safflower oil fatty-acid methyl ester (15 g) characterized in Table 1 was used as the substrate by adding 1,250 ppm of RTE, which corresponds to 500 ppm of polyphenol. The added amounts of the three synergists were 100-10,000 ppm for PE, 100-3,000 ppm for AP, and 100-1,000 ppm for CA, since AP and CA could not dissolve into the substrate at a concentration of more than 3,000 and 1,000 ppm, respectively. In addition, an equimolar amount of each anti-oxidative synergist was also added to the substrates and their synergistic effects on RTE were examined. One thousand two hundred and fifty ppm of RTE, corresponding to 500 ppm of polyphenol, were added to the substrates together with 6.7 × 10−3 mol of the synergists (PE: 3,000 ppm, AP: 1,860 ppm, CA: 940 ppm), according to their solubilities into the substrates. This addition amount corresponded to about 5 times the molar amount of rutin, assuming that the polyphenols contained in RTE consisted of rutin only.

Autoxidation tests were performed in a thermostatic oven at 30°C by adding 1,000 ppm of AIBN to the substrates.

2.6.4 Comparison of the synergistic effects of the three synergists in an emulsifying system
The best synergist for an emulsifying system was sought under the conditions described in 2.6.3, except for the addi-

tion of 1,000 ppm of AAPD to the substrates as the water-soluble radical generator instead of AIBN. Autoxidation was carried out in an incubator (Iwashiya, Ltd., Tokyo, Japan) under constant shaking at 1,000 rpm and 30°C.

2.7 Thermal oxidation testing

The anti-oxidative effects of RTE and Pro on thermal oxidation, which had remained unelucidated, were evaluated, although the basic effects of RTE and Pro on autoxidation of oils and fats have already been reported.

2.7.1 Stability testing

To evaluate the thermal oxidation stability of oils and fats, RTE, Pro, EGCG, PG or Toc was added to the purified soybean oil characterized in Table 1. Each polyphenol content was adjusted to 1,000 ppm, and the stability test was performed on a Rancimat, model 679 (Metrohm Herisau, Ltd., Switzerland) at an air-flow rate of 20 L/h and 120, 140, 160 and 180°C, respectively.

2.7.2 Thermal oxidation testing

To compare the anti-oxidative effect of RTE on the thermal oxidation of oils with that of Toc, thermal oxidation tests were carried out in 200-mL beakers filled with purified soybean oil (60 g), characterized in Table 1, to which 1,250 ppm of RTE, 500 ppm of Toc and 550 ppm of Pro were added as amounts corresponding to 500 ppm of polyphenol. Heating was performed with a cooking heater (Panasonic Corporation, Osaka, Japan) to 180°C, which is the general deep frying temperature, and the heating was continued 8 h per day over a span of 2 days. Four g of each sample were taken every 4 h, and the time course of the oils were monitored by measuring the AV, AnV and PC contents.

Table 1 Characterization of High Linoleic Safflower Oil Fatty Acid Methyl Ester and Purified Soybean Oil.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>High linoleic safflower oil methyl ester</th>
<th>Purified soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA composition (%)</td>
<td>Palmitic acid 7.2</td>
<td>Linoleic acid 75.7</td>
</tr>
<tr>
<td></td>
<td>Stearic acid 2.3</td>
<td>α-Linolenic acid 0</td>
</tr>
<tr>
<td></td>
<td>Oleic acid 14.8</td>
<td></td>
</tr>
<tr>
<td>Peroxide value (PV, me/kg)</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Conjugated diene content (%)</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>Anisidine value (AnV)</td>
<td>—</td>
<td>2.3</td>
</tr>
<tr>
<td>Acid value (AV)</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>Polar compound content (%)</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Polymer content (%)</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Toc content (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3 RESULTS AND DISCUSSION

3.1 Concentration and identification of the anti-oxidants contained in RTE

The components acting as anti-oxidants contained in RTE were concentrated by an alcoholic extraction and silica-gel column fractionation. The polyphenol content was 51.5% for the alcoholic extraction and 88.7% for the silica-gel fractionation. Therefore, it was confirmed that fractionation was much more effective than extraction. Then, the examination of the compounds in the concentrate by TLC analysis revealed two spots clearly on the TLC plate, and it was confirmed that the two components on the plate agreed with the Rf value (Rf = 0.67) of rutin, which is one of the glucosides of quercetin, together with the Rf value (0.84) for quercetin.

These two spots were also identified by FT-IR analysis at 3425 (νO-H), 1655 (νC=O), 1363 and 1168 (δO-H), 1132 (νC–O–C), and 725 (γC–H) cm⁻¹ assigned to rutin and quercetin, and by 1H-NMR analysis in which signals of 1.11 (3H, d, J = 6.1Hz, CH₃), 3.36-3.46 (1H, m), 3.52 (2H, dd, J = 3.2 and 8.8 Hz), 3.79 (2H, d, J = 10.0 Hz), 4.50 (1H, s), 5.10 (2H, d, J = 3.6 Hz, CH₂), 6.21 (1H, s, 2-CH), 6.40 (1H, s, 8-CH), 6.86 (1H, d, J = 8.3 Hz, 6-CH), 6.91 (1H, d, J = 8.3 Hz, 5-CH), and 7.66 (1H, s, 6-CH) ppm were observed.

On the other hand, the standard sample of quercetin gave signals of 6.17 (1H, d, J = 2.1 Hz, C₆-H), 6.37 (1H, d, J =
Therefore, it was considered that the polyphenols contained in RTE were composed mainly of rutin and quercetin, although other polyphenols might exist in RTE.

From the series of results obtained, it was suggested that the anti-oxidative effect of RTE depends on rutin and quercetin, because the RTE concentrate contained those two polyphenols.

3.2 Determination of the polyphenol compounds

The polyphenol content after the preparation of aqueous solutions of polyphenol at optional concentrations was 38.5 ± 1.5% (3.85 ± 0.15 mg EGCG/1 g of sample) for RTE, 88.7 ± 1.8% (8.87 ± 0.18 mg EGCG/1 g of sample) for the RTE concentrate, 49.7 ± 0.9% (4.97 ± 0.09 mg EGCG/1 g of sample) for rutin, 89.3 ± 1.0% (8.93 ± 0.1 mg EGCG/1 g of sample) for quercetin and 90.4 ± 3.0% (9.04 ± 0.3 mg EGCG/1 g of sample) for Pro in term of EGCG, respectively. From these results, it was confirmed that the RTE concentrate contained 2.3 times more polyphenol than RTE and that there are significant differences in polyphenol content between RTE and Pro.

3.3 Evaluation of the DPPH radical-scavenging ability

The radical-scavenging abilities of RTE, RTE concentrates, rutin, quercetin and Pro were evaluated for the DPPH radical. The radical-scavenging abilities of the above compounds were compared by calculation from the calibration curve drawn based on Toc. It was found that the DPPH radical-scavenging ability of RTE was about twice higher than that of Toc when converted to the polyphenol equivalent, as follows: 1.00 for Toc, 2.01 for RTE, 1.87 for the RTE concentrate, 3.50 for quercetin, 4.50 for rutin and 6.58 for Pro, although RTE contained only 38.5% polyphenols. Quercetin and rutin, each of which is considered to be an effective anti-oxidant, were also found to have an anti-oxidizing ability 3.5 and 4.5 times higher, respectively, than Toc.

In addition, it was confirmed that the radical-scavenging ability was not suppressed by silica-gel fractionation, since the anti-oxidizing abilities of RTE and the RTE concentrate were nearly the same.

3.4 Autoxidation testing

3.4.1 Autoxidation testing in a non-aqueous system

To confirm the anti-oxidative effect of RTE due to rutin and quercetin, the time courses of the PV were determined by investigating the autoxidation in a non-aqueous system with substrates containing RTE, RTE concentrate, rutin, quercetin or Toc, as shown in Fig. 1. In this experiment, the time courses of the conjugated diene and residual methyl linoleate contents corresponded to that of the PV. Based on the characteristic changes observed, it was confirmed that the anti-oxidative effects of RTE and the RTE concentrate were nearly the same. Therefore, the anti-oxidative effect of RTE was attributed the rutin and quercetin which are contained in the RTE concentrate. However, rutin showed only a slight anti-oxidative effect, while quercetin surpassed Toc spectacularly in the samples to which standard reagents of rutin or quercetin were added. From these results, it was confirmed that the RTE concentrate contained more rutin than quercetin, as estimated by TLC-FID analysis, because the RTE concentrate is a mixture of rutin and quercetin and the anti-oxidative effect of quercetin was higher than that of the RTE concentrate.

On the other hand, the anti-oxidative effect of the substrate in which Toc and RTE coexisted was equal with the result of adding the effect of Toc to that of RTE, and it was considered that this effect is not synergistic but cumulative.

3.4.2 Autoxidation testing in an emulsifying system

From the comparison of the anti-oxidative effects of RTE and the RTE concentrate, as shown in Fig. 2 which illustrates the time courses of the PVs, there is no difference between the two effects, although more remarkable effects were observed than those described in 3.4.1; that is, RTE and the RTE concentrate displayed greater anti-oxidative effects than rutin, quercetin or Toc in the emulsifying system. Therefore, it was suggested that the synergistic effect in the emulsifying system was derived from the coexistence of rutin and quercetin, since RTE and the RTE concentrate are both mixtures of the two compounds. Furthermore, it was considered that RTE and the RTE concentrate displayed an anti-oxidative effect in the emul-
sifying system because the rutin and quercetin constituting RTE and the RTE concentrate form a uniform system in an emulsifying system more easily than in a non-aqueous system because of their water-solubility.

On the other hand, the anti-oxidative effect of the substrate in which Toc and RTE coexisted was equal to the result of adding the effect of Toc to that of RTE, in the same way as described in 3.4.1, and it was considered that this effect was also not synergistic but cumulative.

3.4.3 Examination of the optimum addition amounts of the three synergists in a non-aqueous system

The optimum addition amounts of PE, AP and CA to RTE were evaluated in non-aqueous autoxidation tests in accordance with 2.6.3.

The systems in which 1,250 ppm of RTE with fixed amounts of the three kinds of synergists and without any synergist were autoxidized and the time ratios of the PVs which reached 100 meq/kg were compared. The time ratios of the samples which contained 1,250 ppm of RTE and to which 1,000 ppm of PE, AP or CA were added were compared with the reference group in which the substrate contained no synergist, and the resulting data were as follows: 1,000 ppm of PE, AP or CA strengthened the antioxidative effect of RTE 2.1-, 1.6- and 1.4-fold, respectively. Therefore, those were concluded to be the optimum amounts to yield the best synergistic effect on RTE. It was also considered that, for RTE, a synergist with a regenerated phenolic hydroxide group was more effective than a metal-scavenging one.

In addition, the synergistic effect of equi-molar amounts \(6.7 \times 10^{-5} \text{ mol}\) of PE (3,000 ppm), AP (1,860 ppm) or CA (940 ppm) on RTE in non-aqueous autoxidation were evaluated in the time course of the PV, as shown in Fig. 3. The time courses of the conjugated diene and residual methyl linoleate contents also corresponded to that of the PV. From the series of results obtained, it was found that RTE coexisting with \(6.7 \times 10^{-5} \text{ mol}\) of AP gave the strongest anti-oxidative effect when equi-molar amounts of synergists were added to the substrates with 1,250 ppm of RTE. In contrast with AP and PE, which are oil-soluble and dissolve easily into the substrate, the water-soluble CA hardly did so. It was considered that the synergist having the higher oil-solubility exhibited the stronger effect under the conditions in which radicals causing lipid oxidation existed in the substrate, since the order of the strength of the synergistic effect was AP > PE > CA.

3.4.4 Comparison of the synergistic effects of the three synergists in an emulsifying system

To investigate the synergistic effects of PE, AP, or CA on RTE in an emulsifying system, autoxidation with equimolar amounts of each synergist was carried out in accordance with 2.6.4. Since the time courses of the PV, conjugated diene content and residual methyl linoleate content suffered corresponding changes, the time courses of the PVs in the AIBN- and AAPD-addition systems were as shown in Figs. 4 and 5, respectively. As is clear from both figures, RTE coexisting with PE had the highest anti-oxidative effect in the AIBN addition system, but the substrate with RTE showed nearly the same effect as the sub-

Fig. 2 Time Course of PV during Autoxidation of High Linoleic Safflower Oil Fatty Acid Methyl Ester in Emulsifying System of Autoxidation.

Fig. 3 Synergistic Effect of Equi-molar Amounts of PE, AP, and CA on RTE in Non-aqueous System of Autoxidation.
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Although AP had an excellent synergistic effect on RTE in the non-aqueous system, only a slight effect was observed in the emulsifying system. On the contrary, PE showed an excellent synergistic effect in both the non-aqueous and the emulsifying system. It was considered that PE exhibited the effect both in the oil and the water phase, since PE showed emulsifying activity.

From those results, it was considered that RTE prevented oxidation in the emulsifying system by scavenging the radicals generated in the aqueous phase.

3.5 Thermal oxidation testing

3.5.1 Stability testing

The stabilities of RTE, Pro, EGCG, PG and Toc against thermal oxidation were compared under heating at 120, 140, 160 and 180°C. The results, as given in Table 2, showed that there were significant differences in the thermal stabilities among soybean oils containing different anti-oxidants as the heating temperature rose.

The order of the strength of each anti-oxidant, by species and temperature, was as follows: PG > Toc > EGCG > Pro > RTE at 120°C, Toc > PG > EGCG > RTE > Pro at 140°C, RTE > Toc > Pro > PG > EGCG at 160°C, RTE > Pro > Toc > PG > EGCG at 180°C.

Although the induction period imposed by each anti-oxidant was shortened with rising heating temperature, the degradation ratio of the control differed according to the anti-oxidant used. Soybean oils with Toc or PG showed excellent thermal oxidation stability compared with soybean oil with RTE at 120 and 140°C. But the stabilities were reversed at 160°C, and the oil with RTE showed the highest stability above 160°C.

When comparing the degradation ratio for each induction period and different heating temperatures, it was found that the ratio at 180°C for Toc was 95% of that at 120°C. On the other hand, it was considered that RTE was more stable against heating than Toc since the degradation ratio for RTE was about 55%. On the basis of these results, it was supposed that the active anti-oxidative components in RTE were either hardly decomposed by heating or exhibited anti-oxidative effects even in their decomposed forms.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heating temperature (°C)</th>
</tr>
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<tr>
<td></td>
<td>120</td>
</tr>
<tr>
<td>Control</td>
<td>1.20</td>
</tr>
<tr>
<td>RTE</td>
<td>1.27</td>
</tr>
<tr>
<td>Pro</td>
<td>1.62</td>
</tr>
<tr>
<td>EGCG</td>
<td>1.65</td>
</tr>
<tr>
<td>PG</td>
<td>6.93</td>
</tr>
<tr>
<td>Toc</td>
<td>4.57</td>
</tr>
</tbody>
</table>

3.5.2 Thermal oxidation testing

Although the results obtained from the time course of the thermal oxidation of soybean oil showed anti-oxidative effects in the order of Toc > Pro ≥ RTE, this result was incompatible with the result from the stability test for thermal oxidation. The reason why RTE and Pro showed little anti-oxidative effect was considered to be that RTE and Pro were only dispersed into the soybean oil, whereas Toc was dissolved uniformly. In addition, it was assumed that the dispersed RTE and Pro formed black sediments upon heating. In the stability test with the Rancimat apparatus, RTE and Pro were dispersed completely in the substrate at all times by aeration at 20 L/h, and it was considered that this situation was very conducive to anti-oxidative activity.

4 SUMMARY

In the present work, the concentration and identification of the active anti-oxidative components contained in RTE were firstly performed, and the concentrate obtained by alcoholic extraction and silica-gel column fractionation were found to account for 51.5% and 88.7% of the polyphenol contents, respectively. Therefore, silica-gel column fractionation was useful for concentrating the active anti-oxidative components of RTE.

Secondly, the active anti-oxidative components in the RTE concentrate obtained by silica-gel column fractionation were confirmed to be rutin and quercetin by TLC, IR and NMR analyses.

Thirdly, the radical-scavenging abilities of RTE and RTE concentrate against DPPH radical were 2.01 and 1.87 times higher than that of Toc against polyphenol, respectively. Therefore, RTE and the RTE concentrate were concluded to have the same radical-scavenging abilities, with no problems arising from the concentration process. In addition, it was confirmed that the radical-scavenging ability of RTE can be attributed to rutin and quercetin.

Fourthly, the anti-oxidative effect of RTE in the autooxidation tests was higher in the emulsifying system than in the non-aqueous system, and its effect was about 2 times higher than that of Toc against thermal oxidation. The synergistic effect on RTE was best shown by hydrogen-radical donor-type synergists, and AP was effective in the non-aqueous system, whereas PE was effective in both the non-aqueous and the emulsifying systems.

Fifthly, RTE and the RTE concentrate showed better thermal stabilities than Toc against thermal oxidation at 160 and 180°C.

From the results described above, RTE can be expected to be used effectively as a new natural alternative for Toc in emulsifying and thermal oxidation systems.

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