Asian Journal of Chemistry; Vol. 24, No. 10 (2012), 4638-4640

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Study on Oxidation Resistance Effect of Catechin Esters

LIN QIANG and HUO QING*

Biochemical Engineering College of Beijing Union University, Beijing 100023, P.R. China

*Corresponding author: Tel: +86 10 52072020; E-mail: huo_q2002@yahoo.com.cn

(*Received*: 18 August 2011; *Accepted*: 9 May 2012) AJC-11455

Catechins were enzymatically esterified with stearic acid by an immobilized lipase Novozym435 in organic solvent (*n*-butanol) at 60 ºC. The reaction time is *ca.* 96 h. Through extraction by column chromatography, the purified catechin esters were collected. The antioxidative activity of catechin esters and catechin had been studied by peroxide value, hydroxyl radicals's clearance and superoxide radical clearance. Peroxide value of catechin esters was the lowest before 48 h, Catechin esters showed better antioxidative activity than the standard antioxidant BHT and other species. The esters had stronger scavenging effect for $^{\cdot}OH$ and $O^{2-\cdot}$ than catechin. So catechin esters can be used as an antioxidant to alleviate the additive oxidation process.

Key Words: Catechin, Catechin esterification content, Peroxide value, Hydroxyl radicals's clearance, Superoxide radical clearance.

INTRODUCTION

The edible oils and their food products on storage show off-odour and sometimes change in colour and taste in the fatty food products. This change occurs as soon as the oils, fats and fatty food products come into contact with atmospheric oxygen. The enzymes and micro-organisms also react and bring about alteration in the structure of oils and fats. This phenomenon of the development of off-flavour, off-odour and change in colour and taste in general is called rancidity[1].

Recent research has concentrated on natural sources of antioxidants such as plant extracts, herbs, spices, seeds and fermentation products. Flavonoids are an interesting group of natural compounds with antioxidants, anticarcinogen, antiviral and antiinflammatory activities. Unfortunately, some of them exhibit low solubility and stability in the oil media. This behaviour can limit their uses efficiently[2]. In view to improve these properties, chemical and enzymatic esterification has been investigated. However, enzymatic approach seemed to be more suitable to these modifications because enzymes are regioselective and the process can be conducted under mild conditions of temperature and pressure[3].

Catechin possesses two benzene rings (called the A- and B-rings) and a dihydropyran heterocycle (the C-ring) with an hydroxyl group on carbon 3. The A ring is similar to a resorcinol moiety while the B ring is similar to a catechol moiety. There are two chiral centers on the molecule on carbons 2 and 3. Therefore, it has four diastereoisomers. Two of the isomers are in *trans* configuration and are called catechin and the other two are in *cis* configuration and are called epicatechin.

The most common catechin isomer is the (+)-catechin. The other stereoisomer is (-)-catechin or ent-catechin. The most common epicatechin isomer is (-)-epicatechin (also known under the names L-epicatechin, epicatechol, (-)-epicatechol, l-acacatechin, l-epicatechol, epi-catechin, 2,3-*cis*-epicatechin or (2R,3R)-(-)-epicatechin)[4].

Green tea has a protective effect against a variety of malignant disorders such as lung cancer, breast cancer and prostate cancer. This preventive potential of green tea against cancer is attributed to the biologically active flavonoids called catechins. Green tea extract and catechin ameliorate chronic fatigue-induced oxidative stress in mice. Evidence is emerging for the role of catechin in the prevention of degenerative diseases such as cancer and cardiovascular disease. Epigallocatechin gallate (EGCG), a major component of green tea, has been previously shown to inhibit platelet aggregation[5].

Keeping in view the above facts, the present investigation was designed to study the antioxidative activity of catechin esters and catechin.

EXPERIMENTAL

Stearic acid, butanol, acetic acid, chloroform, 2,6-di-*tert*-butyl-4-methylphenol (BHT), pyrogallol, $Na_2S_2O_3$, EDTA-2Na-Fe(II), KI, NBT, vitamins E(V_E), hydrogen peroxide, muriatic acid, sodium hydroxide, monopotassium phosphate, mannitol all of them are CP grade, methanol and other reagents are of analytical grade. (Beijing Chemistry Reagent Company); cotton seed oil was obtained from Chaoshifa supermarket. Catechin content ≥ 95 % (Shaanxi Senfu biological technology

Co. Ltd.); safranine T, biochemical reagent (Tianjin Fuchen chemical reagents plant); Lipase Novozym435 (Novzymes (China) Biology Technology Limited Company); molecular sieve (China-America Shanghai Global Molecular sieve Limited Company).

Detection method

Peroxide value determination: Peroxide value is the measure of degree of oxidation or the degree of rancidity. Peroxide value determine the concentration of peroxides in an oil or fat. To determine the peroxide values of cotton seed oils a sample of *ca.* 5 g of cotton seed oil weighed accurately and dissolved in 30 mL of a mixture of acetic acid and chloroform (3:2 v/v). A saturated KI solution (0.5 mL) was added to it. The mixture was allowed to stand for 1 min and then 30 mL of distilled water was added. The whole mixture was titrated with 0.1 N $Na_2S_2O_3$ solution, using 1 mL of 0.5 % starch as an indicator. The peroxide value (POV) was calculated from the relationship given below[6]:

$$POV = V \times N/W \times 1000 \text{ Meq/Kg}$$

where, V = volume of $Na_2S_2O_3$ used, N = normality of $Na_2S_2O_3$, W = weight of the sample.

Clear super oxide anion radical: Pyrogallol in alkaline conditions can release an oxygen free radicals. Super oxide anion radical and NBT form purple compounds which can be measured in the wavelength of 530 nm. The absorption value (A) reflects the content of O^{2-}. Take 0.1 mol/L (pH 8.2) of *tris*-HCl buffer solution 2.5 mL in tube, warm-water bath at 25 ºC 20 min preheat samples, add different concentrations sample 0.2 mL, 0.98 mmol/L NBT 0.6 mL, 10 mmol/L of pyrogallol 0.3 mL, after mixed reaction at 25 ºC water bath 4 min, immediately with 8 mol/L of 0.1 mL HCl to terminate the reaction and the wavelength of 530 nm absorbance was determined at A values[7]. Useful distilled water instead of a blank sample. Determination results in clearance rate.

$$E\ \% = (A_{blank} - A_{sample})/A_{blank} \times 100\ \%$$

Clear hydroxyl radical: ˙OH group is produced by EDTA -Na- Fe(II)-H_2O_2 (Fenton) system. ˙OH can make *Crocus sativus* red T fade, according to the degree of faded with colorimetric method to measure the content ˙OH group. In pH 7.4 reaction system for the solution of the phosphoric acid buffer, *Crocus sativus* red T (520 μg/mL) 0.2 mL, 2 mmol EDTA-2Na-Fe(II) 0.7 mL, different concentrations of selected liquid 1.0 mL, 6 % of H_2O_2 0.4 mL, mixing 0.5 h in 37 ºC water heat. Then measuring absorbance in wave length 520 nm with spectrophotometer[8], experimental results with clearance E %.

$$E\ \% = (A_{sample} - A_{blank})/A_{sample} \times 100\ \%$$

RESULTS AND DISCUSSION

Esterification of catechin with stearic acid: Catechins were enzymatically esterified with stearic acid by an immobilized lipase Novozym435 in *n*-butanol, when ratio between catechins and stearic acid is1:5, adding molecular sieves 4A after 11 h of reaction and the temperature of 60 ºC led to the maximum conversion yield 60.36 %. Through extraction by column chromatography, the purified catechin esters were collected by Varian prosta 210 chromatographic systems. The column of Agilent-C_{18} (30 mm × 250 mm) was used. The mobile phase was composed of methanol:water = 15:85 and the flow rate was 2 mL/min, All HPLC were performed at (30 ± 1) ºC, with UV detection at 278 nm. The structure of catechins stearic acid ester were identified by IR, at around 1700 cm^{-1} carbonyl peaks appeared, indicating that the formation of the fatty acid ester catechins.

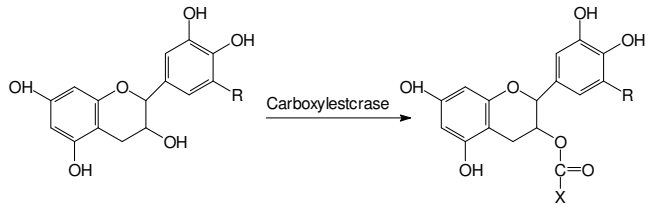

Fig. 1. Enzymatic acylation of catechins by stearic acid catalyzed by immobilized lipase Novozym 435

Catechin esters has smaller polar than catechin, catechin esters will be first separated. So it can be inferred from Fig. 2 that retention time 23 and 24 min are catechin ester peak, retention time 35 min is catechin peak.

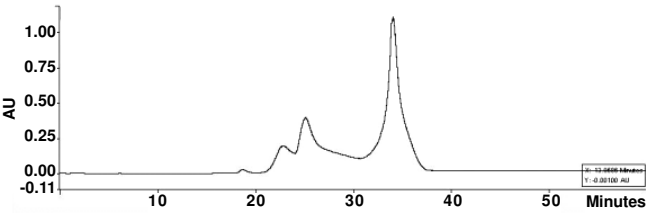

Fig. 2. Catechin esters extraction by column chromatography

On comparing Figs. 3 and 4, 1700 cm^{-1} is carbonyl peaks, indicating the generation of catechin stearate ester.

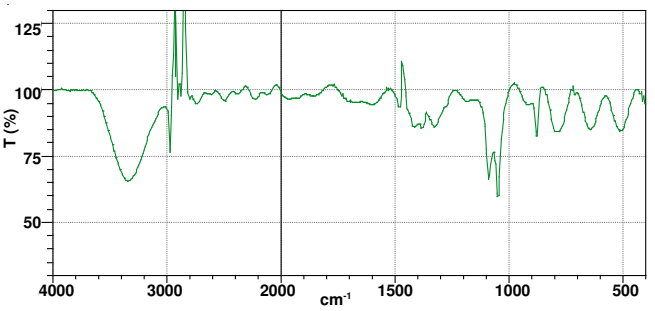

Fig. 3. IR of Catechin

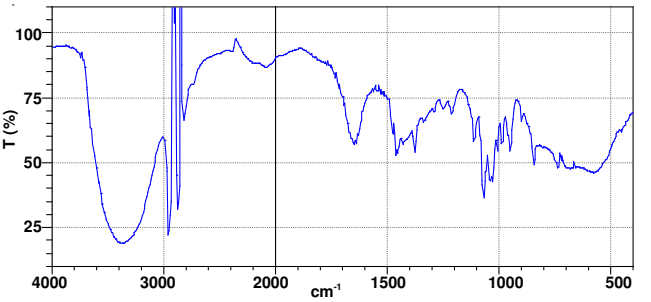

Fig. 4. IR of Catechin esters

Peroxide value determination of catechin esters: According to peroxide value experimental method, the peroxide value of natural flavonoids catechin, catechin stearate ester

and antioxidative activity of standard antioxidant BHT are tested, the results are shown in Figs. 5 and 6.

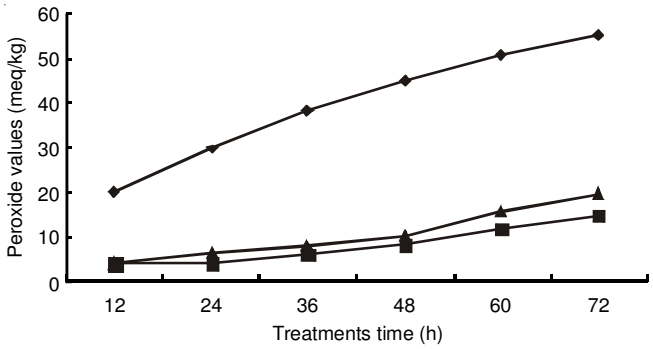

Fig. 5. Antioxidative activity of catechin esters (◆ blank, ▲ BET, ■ catechin esters)

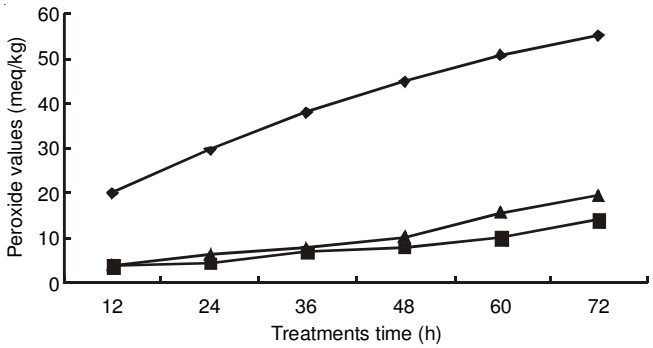

Fig. 6. Antioxidative activity of catechin (◆ blank, ▲ BET, ■ catechin)

From Figs. 5 and 6 we can see, add the same amount, peroxide value of catechin or esterification product is lower than that of the commonly used antioxidants BET. Peroxide value of catechin esterification product is smaller before 48 h, peroxide value of catechin become smaller after 48 h.

Single catechin molecules can provide multiple active hydroxy. Synthetic antioxidants can only provide a single hydroxyl group. Catechins are a mixture of several monomers, monomers of the antioxidant have synergistic effects, which make the antioxidant properties of catechins to be strengthened. Flavonoid glycosides modified by esterification, introduction of long hydrocarbon chain molecules can increase its fat-soluble and the balance of affinities, thus increasing the compatibility with fat and improving oxidation resistance. The solubility of catechin in fat increased over time, so it is observed from the experimental data after 48 h, the antioxidant catechins are also relatively close to its esterification product.

Study on clear hydroxyl radical and super oxide anion radical: Study the clear hydroxyl radical and super oxide anion radical of catechin, catechin stearate ester, vitamin E and BHT.

Table-1 shows that the clear hydroxyl radical and super oxide anion radical of catechin stearate ester are the highest. These experiments also show the catechin stearate ester has the best antioxidant activity.

TABLE-1
CLEARANCE RATE OF $^{\bullet}OH$ AND $O^{2-\bullet}$

Sample	$^{\bullet}OH$ E (%)	$O^{2-\bullet}$ E (%)
Catechin esters	67.35	61.91
Catechin	66.79	60.16
BHT	63.42	50.29
Vit.-E	66.95	51.90

Catechin ester can provide reductive proton like catechins, to capture the process of peroxide generated reactive intermediates radicals. The mechanism of antioxidant is similar to that of vitamin E, BET. Catechin ester structure has reductive the hydroxyl proton which remove free radicals, free radical chain reaction suspend, then stop fat oxidation.

Conclusion

The results of the present investigation indicate that catechin ester and catechol have similar antioxidant activity and they are better antioxidants than BET. Therefore, catechin ester and catechin are better natural antioxidants.

ACKNOWLEDGEMENTS

This work was supported by Biochemical Engineering College of Beijing Union University Subject Construction Projects.

REFERENCES

1. W.O. Lundberg, Autoxidation and Antioxidants, Interscience Pulishers, London, Vol. 2, p. 452 (1962).
2. M. Ardhaoui, A. Falcimaigne, S. Ognier, J.M. Engasser, P. Moussou, G. Pauly and M. Ghoul, *J. Biotechnol.*, **110**, 265 (2004).
3. A. Kontogianni, V. Skouridou, V. Sereti, H. Stamatis and F.N. Kolisis, *J. Mol. Catal. B: Enzym.*, **21**, 59 (2003).
4. M. Arunachalam, M.M. Raj, N. Mohan and A. Mahadevan, *Proc. Indian Nat. Sci Acad.*, **B69**, 353 (2003).
5. H. Nagata, S. Takekoshi, T. Takagi, T. Honma and K. Watanabe, *Tokai J. Exp. Clin. Med.*, **24**, 1 (1999).
6. T. Rana, M.A. Kashmiri and M. Ahmed, *Pak. J. Sci.*, **62**, 67 (2010).
7. R. Zheng, Z.Y. Deng, F.W. Yan, M.X. Gao, C. Zou and M.C. Wu, *J. Nucl. Agric. Sci.*, **21**, 602 (2007).
8. J.Q. Jin and X.L. Bian, *Herb*, **24**, 660 (2001).