Natural Extracts as Potential Source of Antioxidants to Stabilize Polyolefins

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Received 25 November 2009; accepted 2 July 2010
DOI 10.1002/app.33022
Published online 30 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Several natural matrices were investigated as potential sources of antioxidants to be used as plastic additives. Extracts of four matrices obtained under the same experimental conditions were initially considered: green tea, black tea, *Lippia citriodora* and *Hypericum androsaemum*. Both, the antioxidant activity of the extracts and their content in flavanols and quercetin, were compared. The antioxidant activity was determined by DPPH analysis and the phenolic composition by high performance liquid chromatography (HPLC) using ultraviolet (UV) diode array and fluorescence (FL) detectors. Concentration of the flavanols reduced in the same way as their antioxidant activity starting with green tea, through black tea, *Hypericum androsaemum,* and *Lippia citriodora.* The performance of polypropylene samples stabilized with green tea extract, or its individual components catechin and epicatechin, was compared with samples stabilized with a mixture of the synthetic antioxidants Irganox 1076 and Irgafos 168. Each sample was extruded and consecutively reextruded up to four times. The melt flow index (MFI) and the oxidation induction time (OIT) of the samples were measured after each step. The obtained results showed the interest of this natural matrix as a potential source of antioxidants for plastics. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 3553–3559, 2011

Key words: antioxidants; green tea; high performance liquid chromatography (HPLC); poly(propylene); (PP); stabilization

INTRODUCTION

Polymers, and especially polyolefins, need the addition of antioxidants in their formulations to provide protection during processing or fabrication into finished product.[1] Chain breaking antioxidants, sometimes referred to as primary antioxidants, interrupt the first degradation cycle by removing the polymer propagating radicals $ROO^{\bullet}$. Preventive antioxidants, sometimes referred to as secondary antioxidants, interrupt the second oxidative cycle by preventing or inhibiting the generation of free radicals. The most important preventive mechanism is the non-radical hydroperoxide decomposition. Hindered phenols and phosphite esters are important classes of primary and secondary antioxidants, respectively. Because of their complementary antioxidant mechanisms, they are generally used in combination to ensure both highly efficient melt stabilizing systems and long term stability at high service temperatures.[2,3]

The antioxidants and other additives that can be used in the manufacture of plastic materials and articles intended to come into contact with foodstuffs are included in a list of additives established by Directive 2002/72/EEC.[4] During processing or storage additives or their degradation products could migrate from plastic packaging into foodstuffs; therefore, their migration is also regulated by European legislation[4] through Specific Migration Limits (SMLs). In the last years, instead of the synthetic antioxidants usually employed natural antioxidants

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Contract grant sponsor: Xunta de Galicia Govern; contract grant number: PGIDIT05TAL17201PR

Contract grant sponsors: Research University Groups 2007–2009, Xunta de Galicia Govern (Autonomous Community Government); FEDER.

such as α-tocopherol,[5–8] carnosic acid,[9] oregano, savory, and essential oils,[10] carvacrol,[8] or hydroxytyrosol[11] have started to be investigated to reduce the problems associated with the contamination of the food.

In this work, several natural matrices were investigated as potential sources of antioxidants for polyolefins stabilization: green tea, black tea, *Lippia citriodora,* and *Hypericum androsaemum*. *Lippia citriodora* is a herbal species mainly used as a spice and medicinal plant and *Hypericum androsaemum* is a medicinal plant species. These matrices were selected considering their high content in flavonoids, compounds whose high antioxidant capacity has been extensively shown.[12,13]

Finally, the performance of polypropylene stabilized with green tea extract, its individual components catechin or epicatechin, or a mixture of synthetic antioxidants was compared.

The melt flow index (MFI) and oxidation induction time (OIT) of the polypropylene samples was determined after multiple extrusions.

EXPERIMENTAL

Study of the natural plant extracts

Standards and reagents

Methanol (MeOH) was obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA). The phenolic compounds used as references were obtained from the following sources: caffeine, catechin gallate, epigallocatechin, epigallocatechin gallate, gallic acid, gallocatechin gallate, myricetin-3-*O*-rhamnoside, and quercetin-3-*O*-glucoside from Sigma–Aldrich (Steinheim, Germany); catechin, epicatechin, epicatechin gallate, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-rutinoside from Extrasynthése (Genay, France); Irgafos 168 (tris(2,4-di-*tert*-butylphenyl)-phosphate) and Irganox 1076 (octadecyl-3-(3,5-di-*tert*butyl-4-hydroxyphenyl)-propionate) from Ciba (Basel, Switzerland).

Plant extracts preparation

Aqueous extract. About 3.0 g of each dried powdered sample were boiled for 15 min in 300 mL of water and then filtered through a Büchner funnel. The resulting extract was lyophilized in a Labconco 4.5 Freezone apparatus (Kansas City, MO). A yield of 0.9–1.1 g based on dry-ash free basis was obtained.

Methanolic extract. About 3.0 g of dried powdered sample was mixed with 300 mL of methanol under stirring for 15 min at 30°C and then filtered through a Büchner funnel. The resulting extract was evaporated to dryness under reduced pressure at 30°C. A yield of 0.57 g based on dry-ash free basis was obtained.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antiradical activity of the extracts was determined spectrophotometrically in an ELX808 IU Ultra Microplate Reader (Bio-Tek Instruments), by monitoring the disappearance of DPPH at 515 nm, according to a described procedure.[14] For each extract, a dilution series composed of five different concentrations was prepared in a 96 well plate. The reaction mixtures in the sample wells consisted of 25 μL of aqueous extract and 200 μL of DPPH dissolved in methanol. The plate was incubated for 30 min at room temperature. Three experiments were performed in triplicate.

Chromatographic analysis

Qualitative analysis by HPLC-UV. The extracts were analyzed on an analytical HPLC unit (Gilson) with a photodiode array detector, using a Spherisorb ODS2 column (25.0 cm × 0.46 cm; 5-μm particle size Waters, Mildford, MA) with a C_{18} ODS guard column. The system solvent used was a gradient of water/formic acid (19 : 1) and methanol.[15]

Quantitative analysis by HPLC-UV-FL. It was carried out using a Waters Alliance 2695 system equipped with a quaternary pump, autosampler, a Waters 996 photodiode array detector, and a Waters 2475 fluorescence detector. Chromatographic separation was performed on a reversed-phase SunFire C_{18} analytical column (3.0 × 150 mm^2, 3.5 μm Waters). Reverse phase chromatography with methanol : water was used as mobile phase.[16]

Study of the use of green tea extract as antioxidant in polypropylene

Green tea extract

Several dried organic green tea samples were accurately weighed (2 g ± 0.0001 g), added in an Erlenmeyer flask and extracted in a sonication system with 20 mL of methanol. The methanolic extracts were mixed, filtered and then, concentrated to dryness under reduced pressure (30°C, 200 mbar). A viscous liquid was obtained and stored at 4°C until its use.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed using a Perkin–Elmer TGA-7 microbalance coupled to a 1022 Perkin–Elmer microprocessor. The microbalance

TABLE I
Properties of the Additives Used and Concentrations Added to the PP Samples

Sample	Additive	Melting point (°C)[a]	Boiling point (°C)[17]	Thermooxidative stability (°C)[b]	Concentration (%)
1	Nonstabilized PP				–
2	Green tea extract				0.05
3	Irgafos 168	183–186	594.2 ± 50.0	259	0.1 of each
	Irganox 1076	50–55	568.1 ± 45.0	276	compound
4	(+)-Catechin	175–177	629.2 ± 55.0	227	0.05
5	(−)-Epicatechin	240	629.2 ± 55.0	265	0.05

[a] Melting point values were provided by the corresponding commercial sources.
[b] Thermooxidative stability was measured at 5% mass loss.

was calibrated making use of the Curie points of perkalloy and nickel. Dynamic experiments were conducted under oxygen atmosphere. The heating rate was 10°C min^{-1}. The temperature range of the experiments was from room temperature to 700°C. Catechin was heated at 100°C during 4 h prior to the test.

Polymer processing

The polymers containing antioxidants were extruded using a Brabender DSE 20 double screw extruder with five heating zones with the following zone temperature settings: 200/200/200/200/200/200°C and a die head temperature of 210°C; length/diameter (L/D): 40; screw speed: 35 rpm.

The polypropylene used was REPSOL PP044 W3F (commercially stabilized with a little amount of Irgafos 168), an homopolymer with MFI: 3.0 g/10 min (230°C; 2.16 kg) (ISO 1133).

Polypropylene was mixed with the corresponding concentration of antioxidants according to Table I.

A sample of pellets of the extruded polymer from the first pass was taken out for melt flow index (MFI) and oxidation induction time (OIT) measurements. The remaining polymer sample was re-extruded under the same conditions up to four times with polymer sampling after each pass for further analysis.

Assessment of thermal stability

Melt flow index (MFI) was measured using a CEAST melt flow tester at 230°C (2.095 mm × 8 mm die, 2.16 kg). The obtained results are mean of three measurements.

Oxidation induction time (OIT) was measured on a Perkin–Elmer serie 7 DSC isothermally at 200°C under inert atmosphere, which was subsequently switched to oxygen atmosphere. Analyses were carried out according to EN 728. The obtained results are mean of two measurements.

RESULTS AND DISCUSSION

Comparison of antioxidant activity of plants extracts

The antioxidant capacity of extracts obtained from four natural matrices: green tea, black tea, *Lippia citriodora,* and *Hypericum androsaemum* was measured by the DPPH assay. The DPPH assay constitutes a screening method currently used to provide basic information about the antiradical activity of extracts. Reduction of DPPH by antioxidants leads to a loss of absorbance at 515 nm.[13] The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC_{50}. Aqueous extracts of each matrix were prepared under similar experimental conditions. Both tea extracts showed higher antioxidant capacity (IC_{50} = 9 μg mL^{-1}) than *Lippia citriodora* (IC_{50} = 31 μg mL^{-1}) and *Hypericum androsaemum* (IC_{50} = 23 μg mL^{-1}) (Fig. 1). A methanolic extract of green tea was also prepared showing IC_{50} = 9.6 μg mL^{-1}.

Analysis of the extracts by HPLC

First, the qualitative phenolic profile of the selected matrices was considered. Phenolic profile of the extracts of *Lippia citriodora* and *Hypericum androsaemum* has been previously reported[18,19] with high content in flavonols. Both tea extracts, green and black, were analyzed by HPLC with a UV diode array detector. 12 compounds were identified (Fig. 2 and Table II): gallic acid, caffeine, 5 flavanols (catechin, epigallocatechin, epigallocatechin gallate, epicatechin, epicatechin gallate) and 5 flavonols (myricetin glycoside, quercetin glucoside, quercetin rutinoside, kaempferol glucoside, kaempferol rutinoside). As it was expected, the most abundant compounds seem to be the flavanols.

According to Vinson et al.[12] flavanols have higher antioxidant activity than flavonols, and quercetin is the flavonol that shows to be the best antioxidant. Therefore, the second part of the chromatographic study was focused on the determination of flavanols

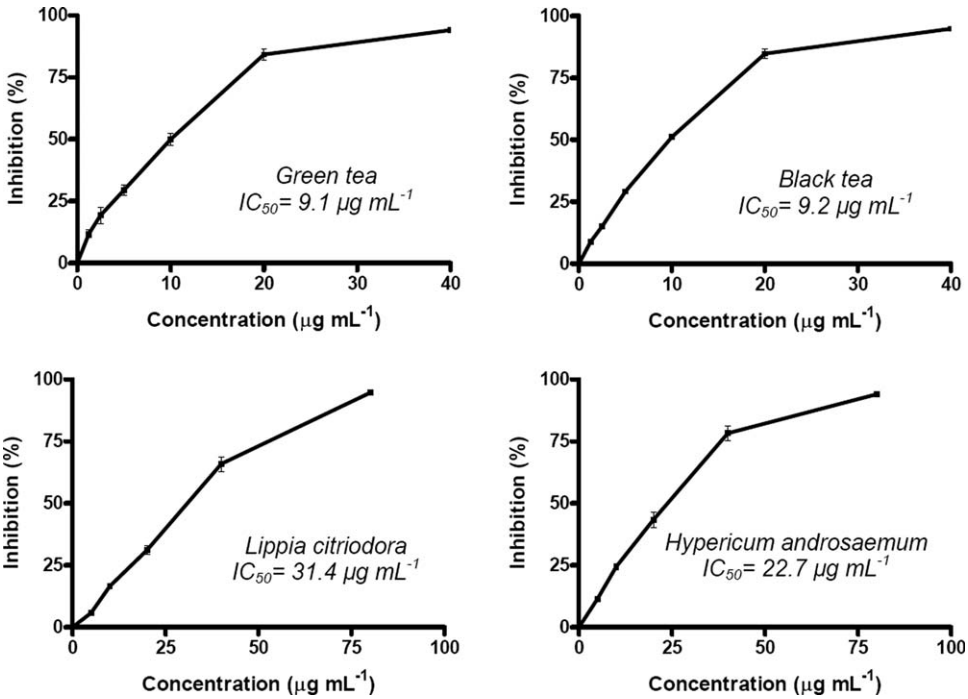


Figure 1 Effect of the extract on DPPH reduction. Values show mean ± standard deviation from three experiments performed in triplicate (aqueous extract).

and quercetin in the considered extracts using a HPLC method with two detectors, UV diode array (Fig. 3) and fluorescence (FL) (Fig. 4). Fluorescence detector allowed determining epigallocatechin free of the interferences that appeared with the UV detector. Moreover, it improved the sensitivity of the method for some compounds such as catechin and epicatechin.

Concentration of the flavanols decreased starting with green tea, through black tea, *Hypericum androsaemum,* and *Lippia citriodora* (Table III). Concentration of quercetin was much lower and could be only quantified in green tea and *Hypericum androsaemum*.

Green tea extract was selected to be tested as an antioxidant for polypropylene, considering that it showed both the highest content in flavanols and the highest antioxidant capacity. Moreover, its individual components catechin and epicatechin were also chosen to be added to polypropylene, due to their higher stability compared with the other flavanols.[20,21]

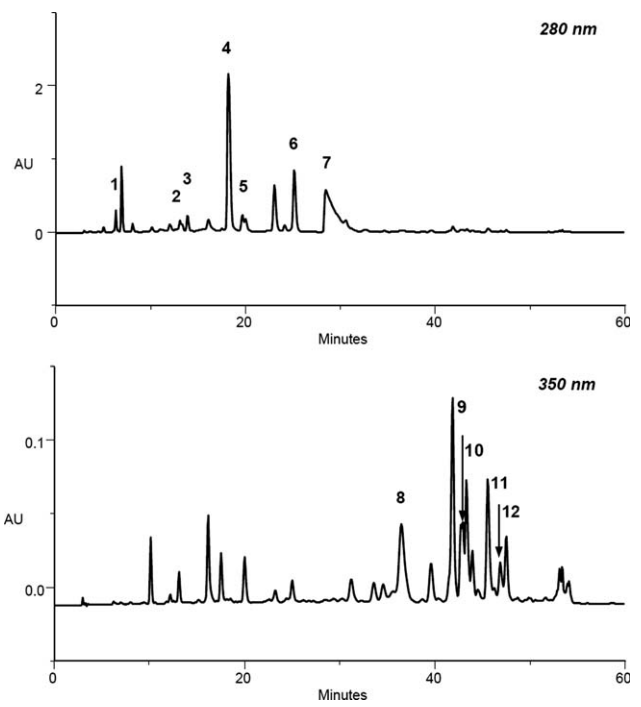


Figure 2 Initial identification of the green tea extract by HPLC-UV. Identification of the peaks according to Table II.

TABLE II
Identified Compounds in Tea Extracts by HPLC-UV

Peak	Compound	Wavelength
1	Gallic acid	280 nm
2	Catechin[a]	
3	Epigallocatechin	
4	Epigallocatechin gallate	
5	Epicatechin	
6	Epicatechin gallate	
7	Caffeine	
8	Myricetin glycoside	350 nm
9	Quercetin glucoside	
10	Quercetin rutinoside	
11	Kaempferol glucoside	
12	Kaempferol rutinoside	

[a] Peak is not pure.

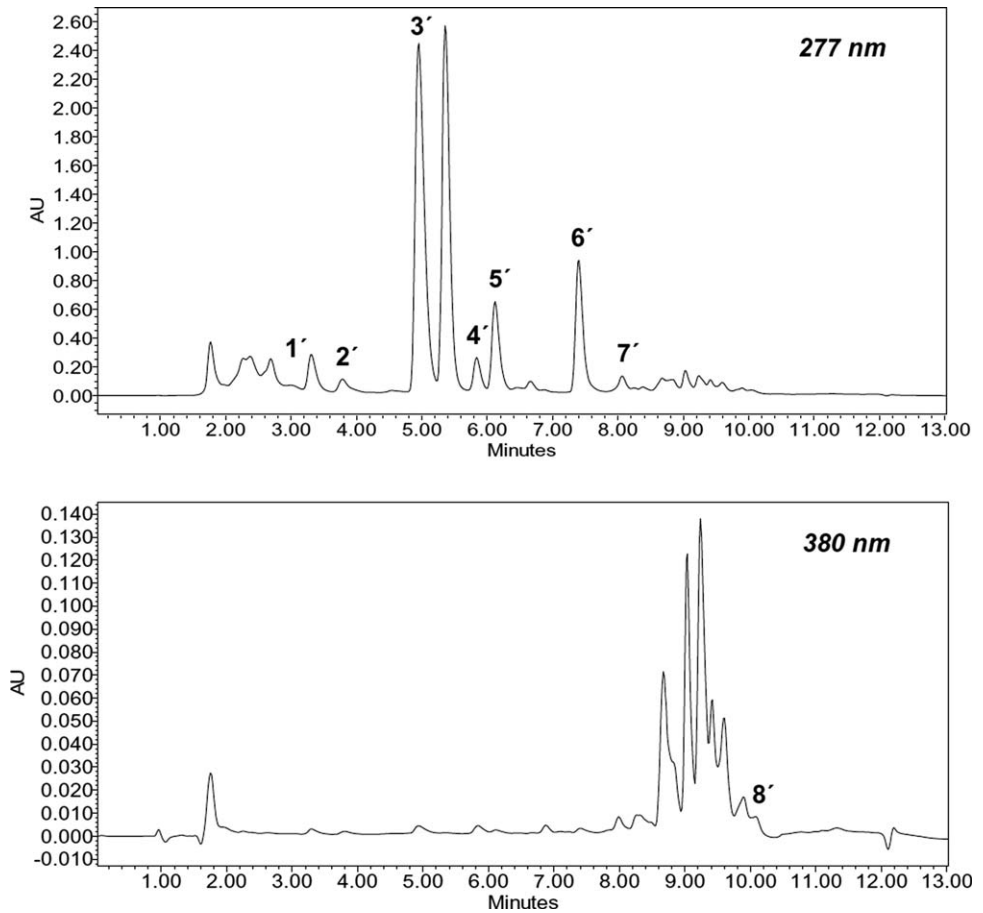


Figure 3 Identification and quantification of flavanols and quercetin in the green tea extract by HPLC-UV. Identification of the peaks according to Table III.

Use of green tea extract as additive of polypropylene

To evaluate the performance of green tea extract as an antioxidant for polypropylene several samples were prepared: stabilized directly with the green tea extract, with its individual components catechin or epicatechin, and with the mixture of synthetic antioxidants Irganox 1076 and Irgafos 168. They were also compared with a nonstabilized sample (Table I).

To enable the comparison between the samples, the same processing conditions were used for all of them (see above). They were selected considering the properties of the polypropylene and the tested antioxidants (Table I).

As expected, the MFI of each stabilized sample was generally lower than the one corresponding to the nonstabilized PP (Fig. 5). Initial MFI values were similar for all the samples, but higher the number of extrusion passes, higher were the differences observed. By comparing the MFI after four extrusion passes with its initial value for each sample, the highest increase was showed by nonstabilized PP (21%). The rest of the MFI increases provided the following order: Irganox 1076+ Irgafos 168 (12%) > epicatechin (11%) > tea extract (7.8%)> catechin (3.5%). Therefore, formulations containing natural antioxidants have provided better melt flow property after consecutive processing compared with the commercially tested antioxidants and the nonstabilized PP.

To further asses the oxidation stability of the samples, oxidation induction time (OIT) was also measured by DSC (Fig. 6). The longest OIT obtained for catechin and epicatechin confirmed that these compounds provided

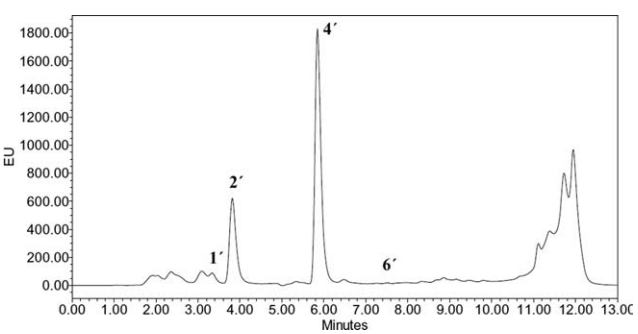


Figure 4 Identification and quantification of flavanols in green tea extract by HPLC-FL. Identification of the peaks according to Table III.

TABLE III
Concentration of Flavanols and Quercetin in Natural Extracts Determined by HPLC-UV-FL

$mg_{compound}\ g^{-1}_{sample}$

		Green tea		Black tea		*Hypericum androsaemum*		*Lippia citriodora*	
Peak		PDA	FL	PDA	FL	PDA	FL	PDA	FL
1′	(−)-Epigallocatechin	*	29.2	*	4.95	nd	nd	nd	nd
2′	(+)-Catechin	3.47	2.89	0.93	0.67	0.95	0.58	nd	nd
3′	(−)-Epigallocatechin gallate	72.0	nd	13.3	nd	nd	nd	nd	nd
4′	(−)-Epicatechin	5.5	5.23	0.75	0.96	0.49	0.46	nd	nd
5′	(−)-Gallocatechin gallate	10.5	nd	1.37	nd	nd	nd	nd	nd
6′	(−)-Epicatechin gallate	13.1	10.3	4.84	4.59	nd	nd	nd	nd
7′	(−)-Catechin gallate	0.98	nd	0.31	nd	nd	nd	nd	nd
	Total flavanols	105	47.6	19.8	11.2	1.45	1.04	nd	nd
8′	Quercetin	0.043	nd	nq	nd	0.71	nd	nd	nd

* : Peak not pure; nd: not detectable; nq: not quantificable.

polypropylene with stabilization against thermal-oxidation degradation. It is worth to remark that the better performance achieved using natural antioxidants compared with the synthetic ones was obtained using lower amounts of additives (Table I).

The mechanism of action of the synthetic antioxidants used is already known (Fig. 7).[3,22] Regarding catechins, although their high antioxidant capacity has been extensively shown, little is known about their antioxidative mechanisms. According to Bors et al.,[23] catechins satisfy one of the criteria for effective radical scavenging: the *o*-dihydroxy structure of their B ring (Fig. 8) which confers higher stability to the radical form and participates in electron delocalization for effective radical scavenging. But in the last years other possibilities to explain the effectiveness of catechins have been proposed. Therefore, in a theoretical study about the chemical reactivity properties of (+)-catechin and (−)-epicatechin Mendoza-Wilson and Glossman-Mitnik[24] found that the preferential sites for radical attack would be the C6 of the ring A and the H4′ (hydroxyl group) of the ring B. On the other hand, Kondo et al.[25] carried out a study to try to elucidate the antioxidative mechanisms of catechins that showed their affectivity in scavenging peroxyl radicals both in a liposomal system and in an aqueous system. The authors proposed tentative antioxidative mechanisms of catechins depending on the experimental results and theoretical calculations that suggest that hydrogen at the C-2 position may be abstracted by free radicals (Fig. 8). Moreover, they found out that the compound produced from epicatechin by radical oxidation can also function as an antioxidant and, as a result, epicatechin has a longer inhibition period. This last one may explain the better performance of the material containing catechin or epicatechin after multiple extrusions.

CONCLUSIONS

1. Extracts of green and black tea showed higher antioxidant capacity than other considered plants: *Lippia citriodora* and *Hypericum androsaemum*.

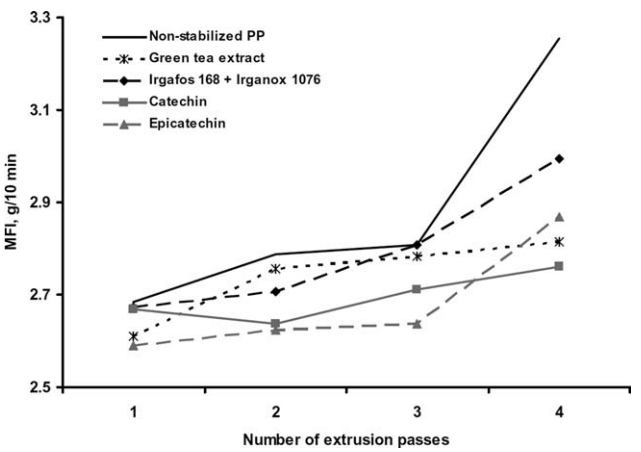


Figure 5 Stability of PP with different antioxidants or green tea extract: MFI measurements ($n = 3$).[26]

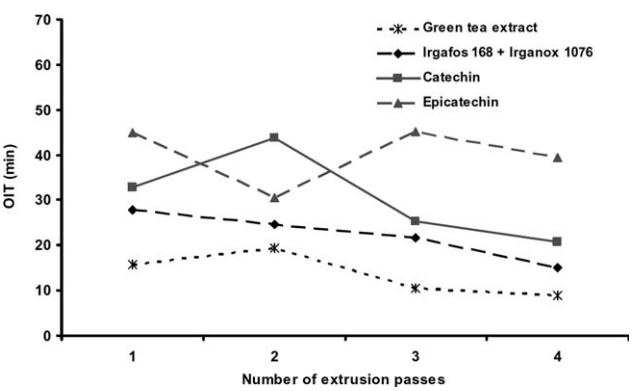
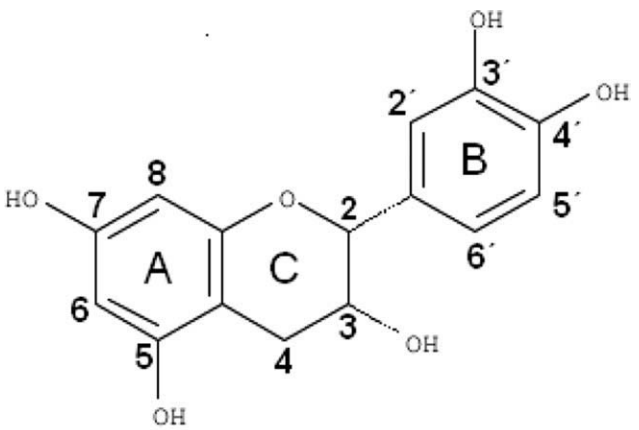


Figure 6 OIT of PP containing different antioxidants or green tea extract as measured by DSC ($n = 2$).

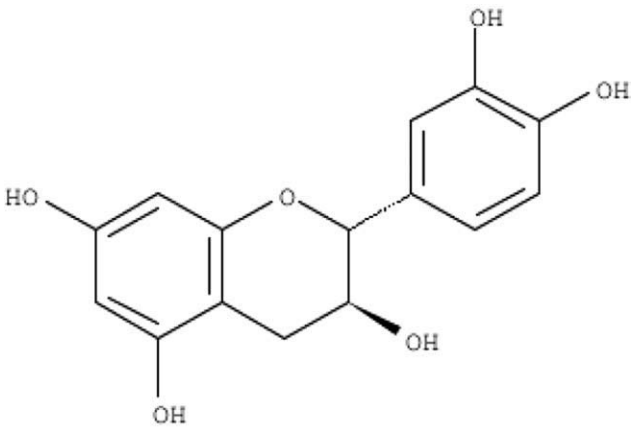
Hindered phenols antioxidants
$ROO^{\bullet} + Ar\text{-}OH \rightarrow ROOH + Ar\text{-}O^{\bullet}$ $ROO^{\bullet} + Ar\text{-}O^{\bullet} \rightarrow$ Inactive products
Aryl phosphite antioxidants
$(OR)_3P + ROOH \rightarrow (OR)_3P{=}O + ROH$
$(OPh)_3P + ROO^{\bullet} \rightarrow [ROOP^{\bullet}(OPh)_3] \rightarrow (OPh)_3P{=}O + RO^{\bullet}$
$(OPh)_3P + RO^{\bullet} \rightarrow [ROP^{\bullet}(OPh)_3] \rightarrow (RO)(OPh)_2P + PhO^{\bullet}$
$PhO^{\bullet} + ROO^{\bullet} \rightarrow$ Inactive products

Figure 7 Scheme of the mechanism of action of antioxidants.[3,22]

2. Flavanols and quercetin were quantified in the selected extracts by HPLC-UV-FL, considering that these phenolic compounds theoretically show the highest antioxidant capacity. Their content in flavanols decreased in the same order than their antioxidant activity.



(-) epicatechin



(+) catechin

Figure 8 Structures of (+) catechin and (−) epicatechin.

3. The possibility of using as antioxidant either an extract of green tea or their individual components catechin or epicatechin in polypropylene was tested. To evaluate the stabilization due to the presence of additives the melt flow index and the oxidation induction time of the samples were measured after multiple extrusions. The stability of the material was comparable to the one stabilized with synthetic antioxidants, showing the interest of this matrix as a potential source of natural antioxidants for plastics.

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