Catechin Transformation As Influenced by Aluminum

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Polyphenols (catechins) are vital biomolecules in tea plants (*Camellia sinensis*), which are well-known as typical Al accumulators. However, the interaction between Al and catechin remains obscured. The objective of the present study was to investigate the effect of Al on the transformation of (+)-catechin. Solutions with OH/Al molar ratios of 2.5 (pH 5.5) and 3.0 (pH 7.0) prepared at Al/catechin molar ratios (*R*) of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 were aged for 7 and 30 days, respectively. The precipitates were collected and examined by wet chemistry, X-ray diffraction, transmission electron microscopy, electron spin resonance (ESR), cross-polarization magic angle (CPMAS) ^{13}C nuclear magnetic resonance (^{13}C NMR) analyses, and Fourier transformation infrared absorption spectrometry (FT-IR). The weight of the precipitates increased with increasing Al/catechin molar ratios and with prolonged aging. The molar ratios of Al/catechin in the precipitates increased with increasing initial Al/catechin molar ratios and were close to the initial solution Al/catechin molar ratios. The chemical analysis and spectroscopic studies indicated that Al was bonded with catechin, forming a 1:1 type complex. The reaction of crystalline catechin with Al resulted in the formation of X-ray noncrystalline precipitates. The solid-state CPMAS ^{13}C NMR spectra of the precipitates show the change in chemical shifts of catechin as a result of catechin complexation with Al. The FT-IR spectra of the Al−catechin precipitates also show the loss of absorption bands of several functional groups compared with catechin. The FT-IR data substantiate this reasoning. The ESR spectra of the precipitates show a single symmetrical line devoid of any fine splitting, indicating the presence of free radicals of semiquinones, which are commonly present in humified materials.

KEYWORDS: Aluminum; (+)-catechin; complexation; coprecipitation; polymerization; semiquinones

INTRODUCTION

Aluminum (Al) is generally recognized as a plant toxin, but the tea plant (*Camellia sinensis*), which is well-known to be a typical Al accumulator, grows well on very acidic soil containing high levels of soluble Al ions (*1*). Tea plants are always cultivated in acid red soils and foggy zones (i.e., 800−1200 m in elevation on forest soils) in subtropical regions (*2*). Tea takes up Al throughout its life due to the high solubility of Al in acid soils and can accumulate great quantities of Al in tea leaves, ranging from 8700 to 23000 mg kg^{-1}, and even up to 30000 mg kg^{-1} in old leaves (*3*). Thus, Al content significantly increases with age of the leaf (*4, 5*). In explaining the internal resistance to Al, it has been suggested that organic acids and polyphenols could detoxify Al by chelation (*6*). It has also been demonstrated that a substantial quantity of polyphenols was found in the endodermis and that a considerable amount of Al was sequestered in the cell wall (*7*).

The chemical composition of tea is complicated, containing polyphenols, alkaloids (i.e., caffeine, theophylline, theobromine, etc.), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals and trace elements, and other undefined compounds (*8*). Among these, the polyphenol constitutients are the most interesting group of tea leaf components and exhibit potential antioxidant activity in vitro and in vivo (*9*). Catechins are one of the six classes of flavonoids and are the principal components of green tea polyphenols. Catechins, namely, (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG), have recently received much attention as protective agents against cardiovascular disease and cancer (*10*). It is generally believed that catechins, the principal bioactive compounds in tea, are responsible for the claimed therapeutic activity of tea. Polyphenols have high reactivity with other phenolic or metal compounds, giving rise to compounds with complicated structures. These transformations affect the sensorial properties such as color and taste (*11*).

The products of Al hydrolysis play significant roles in terrestrial and aquatic environments, particularly through their binding of nutrients and pollutants (*12−18*). Therefore, extensive investigations have been conducted to study the ionic factors affecting the hydrolytic reactions of Al. When Al salt solutions are partially neutralized with base, the hydrolysis of Al results in the initial formation of amorphous and poorly crystalline aluminum hydroxide solids, which will subsequently transform

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into crystalline aluminum hydroxides upon aging (*18, 19*). There are three polymorphs of aluminum hydroxides, that is, gibbsite, bayerite, and nondstrandite (*18*). To understand the formation mechanism of aluminum hydroxide, there have been extensive studies on the precursors of aluminum hydroxides existing in the conditions, where Al is partially neutralized (OH/Al molar ratios $\leq$ 3) (*20, 21*). However, the effect of catechins, which are vital biomolecules in tea plants, on the transformation of Al and the effect of Al on the transformation of catechin remains to be uncovered. Therefore, the interaction between Al and catechin is of fundamental significance in understanding the transformation of Al and catechin. The objective of the present study was to investigate the effect of Al on the transformation of (+)-catechin.

MATERIALS AND METHODS

Materials. (+)-Catechin was obtained from Sigma Chemical Co. (St. Louis, MO) without further purification. Aluminum chloride was purchased from Merck (DAC, USP). Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA) prior to use.

Sample Preparation: Effect of Al on Catechin Transformation. An aliquot of 125 mL of 4×10^{-3} M (+)-catechin solution was mixed with 50 mL of $AlCl_3$ solution (concentration ranging from 1×10^{-2} to 2×10^{-3} M) to fix the Al/catechin molar ratios (*R*) at 0, 0.2, 0.4, 0.6, 0.8, and 1.0. The samples were titrated to pH 5.5 and 7.0 with 0.1 M NaOH at a rate of 1 mL/min at 25 °C using a TIM 865 titroprocessor in the set mode, diluted to 250 mL, and then aged at 25 °C for 7 and 30 days. The pH and turbidity (nephelometric turbidity unit, NTU) of the suspensions as the end of each aging period were measured using a Radiometer pH M83 pH-meter and Hach ratio/XR turbidity meter, respectively. The solution was obtained after passage through a 0.1 μm Millipore filter for visible absorption measurements. Visible absorption spectroscopy was employed to monitor the humification of catechin by measuring changes in absorbance of the supernatant of the reactions systems in the visible range from 400 to 665 nm (*22*).

The suspensions were then centrifuged at 17700*g* for 10 min to separate the supernatants from the precipitates. The precipitates were washed with deionized distilled water, then freeze-dried, and preserved in a refrigerator (4 °C). However, the control samples (only catechin or Al) were transferred to dialysis tubes with a molecular weight cutoff of 3000 Da to dialyze against deionized distilled water to dialyze the fractions of ion (Na^+, Cl^-), then freeze-dried, and preserved in a refrigerator. The dialysis method was used instead of centrifugation for the control sample because the control sample could not be collected by centrifugation.

Analysis of the Precipitates. *Total Al Analysis.* The total Al in the precipitates was determined after digestion with a mixture of HNO_3 and HCl (*23*).

Organic C Analysis. The organic C content of the precipitates was determined by using a CHN analyzer (Carlo Erba nitrogen analyzer).

X-ray Diffraction (XRD) Analysis. The precipitates were examined by XRD using Cu Kα radiation on a Rigaku Miniflex X-ray diffractometer equipped with a graphite monochromator, operating at 35 kV and 15 mA at a step-scan of 0.02° 2θ/s. The X-ray patterns were recorded in the range of 3–50° 2θ.

Transmission Electron Microscopy (TEM) Analysis. One drop of suspension was dried on carbon-coated film on copper grids (3.05 mm in diameter) and then examined under a JEOL TEM (JEM 100 CXII) operated at 100 kV for selected area electron diffraction (SAED) and bright and dark field images of particles.

Electron Spin Resonance (ESR) Spectroscopy Analysis. The ESR spectra of the precipitates were recorded at ambient temperature on a Bruker B-ER 418S spectrometer, employing 100 kHz modulation and a normal frequency of 9.76 GHz. Line widths and *g* values were calculated according to standard methods (*24*).

Cross-Polarization Magic Angle (CPMAS) 13C Nuclear Magnetic Resonance (NMR) Analysis. Approximately 300 mg of sample was packed into 7 mm diameter silicon nitride rotors with Kel-F packs. CPMAS 13C NMR spectra of the precipitates were obtained at ambient

temperature on a Bruker DMS400 spectrometer with a CPMAS.AU accessory at 90.561 MHz using 80 Hz line broadening, with a 90° pulse of 6.5 μs and a recycle delay of 1 s, and a spinning speed of 4 kHz. Tetramethylsilane (TMS) was used as a reference.

Fourier Transformation Infrared Absorption Spectrometry (FT-IR) Analysis. One milligram of the Al–catechin precipitates dried in a vacuum desiccator was ground and mixed thoroughly with 200 mg of oven-dried KBr powder of analytical regent (Merck, DAC, USP). The powder was placed in a die and compressed into a transparent disk. The infrared spectra were recorded by using a Bio-Rad FTS-7 double-beam infrared spectrophotometer.

RESULTS AND DISCUSSION

Color Development and Turbidity of Aging Samples. The samples prepared at different pH values, OH/Al molar ratios, and Al/catechin molar ratios and aged for 7 and 30 days can be grouped into three categories on the basis of their appearances: clear sol, slightly turbid, and coarse precipitates (**Table 1**). At both pH 5.5 or 7.0, the sample appearance changed with Al/catechin molar ratios of the sample preparations. In general, the samples progressively appeared from slightly turbid to coarse precipitates as the Al/catechin molar ratios increased. The turbidity of the samples prepared at pH 5.5 was quite different from that of the samples prepared at pH 7.0, but there were no significant differences at 7 and 30 days of aging. The color development of samples prepared at pH 5.5 and 7.0 showed significant differences between the control and Al–catechin samples. The darkening of the Al–catechin system is attributed to the complexation of catechin with Al and the resultant browning (humification) of catechin. Turbidity in the control samples of Al (blank) aged for 30 days was higher than that of the samples aged for 7 days. This is because aluminum can be slowly hydrolyzed to form suspended aluminum hydroxides (*17*). The absorbance of the solutions (<0.1 μm) at 472 and 664 nm shows the influence of absorption values with increasing pH, aging period, and especially Al to catechin molar ratios (**Table 2**), indicating that these factors enhanced humification of catechin. The absorbances at these wavelengths have been commonly used to study humification of polyphenols (*25*).

pH of Suspensions. At the end of the reaction period, the final pH values of the suspensions of the samples studied were lower than the initial pH values (**Table 3**). This is attributed to the hydrolysis of Al and polymerization of catechin, which generate H^+ in the solution. The pH of the reaction system at OH/Al molar ratios of 3.0 (pH 7.0) after aging for 7 and 30 days increased with increasing Al/catechin molar ratios, but such a trend was not evident in the system at OH/Al molar ratios of 2.5 (pH 5.5). On the other hand, at the end of 7 and 30 days in the absence of catechin or Al (control) the suspension showed a lower pH than those of Al–catechin samples (**Table 3**). This is attributed to not only Al complexing with catechin but also the partial neutralization of hydrolytic reactions of Al.

Dry Weight of Precipitates after Aging. After 7 and 30 days of aging, the suspensions were centrifuged at 17700*g* for 10 min to separate the precipitates from supernatants. The dry weight of the precipitates of the reaction system at OH/Al molar ratios of 2.5 and 3.0 increased with increasing Al/catechin molar ratios and with prolonged aging (**Table 4**). It could only collect a few dry precipitates at an Al/catechin molar ratio (*R*) of 0.2, but there were no detectable precipitates in control samples (only catechin or aluminum). The dry weights of samples having an OH/Al molar ratio of 2.5 were slightly higher than those of samples having an OH/Al molar ratio of 3.0.

Al and Organic C Contents in Precipitates. At OH/Al molar ratios of 2.5 (pH 5.5) and 3.0 (pH 7.0), the percentage of

Table 1. Appearance, Color Development, and Turbidity of Samples Prepared at Different Al/Catechin Molar Ratios and after Aging for 7 and 30 Days

	OH/Al = 2.5 (pH 5.5)		OH/Al = 3.0 (pH 7.0)	
	7 days	30 days	7 days	30 days
AlCl$_3$ (blank)	slightly turbid [3.30 ± 0.05]^a white (7.5YR 8/1)^b	slightly turbid [3.80 ± 0.29] white (7.5YR 8/1)	slightly turbid [22.4 ± 0.05] gray (5YR 8/1)	slightly turbid [26.7 ± 1.21] gray (5YR 7/1)
R = 0^c	clear sol [0.39 ± 0.05] light yellow (5Y 7/6)	clear sol [0.39 ± 0.03] light yellow (5Y 7/8)	clear sol [0.32 ± 0.02] dark yellow (5YR 6/8)	clear sol [0.33 ± 0.03] dark yellow (5YR 5/8)
R = 0.2	slightly turbid [7.50 ± 1.01] brown (7.5YR 7/1)	slightly turbid [9.00 ± 1.10] brown (7.5YR 3/1)	lightly turbid [5.30 ± 1.04] dark brown (5YR 5/6)	slightly turbid [5.20 ± 1.04] dark brown (5YR 3/1)
R = 0.4	coarse precipitate brown (7.5YR 7/1)	coarse precipitate brown (7.5 YR 5/6	coarse precipitate dark brown (5YR 5/8)	coarse precipitate dark brown (5YR 3/1)
R = 0.6	coarse precipitate brown (7.5YR 7/1)	coarse precipitate brown (7.5YR 6/6)	coarse precipitate dark brown (5YR 4/1)	coarse precipitate dark brown (5YR 3/1)
R = 0.8	coarse precipitate brown (7.5YR 7/1)	coarse precipitate brown (7.5YR 7/6)	coarse precipitate dark brown (5YR 4/1)	coarse precipitate brown (5YR 3/1)
R = 1.0	coarse precipitate brown (7.5YR 7/1)	coarse precipitate brown (7.5YR 7/4)	coarse precipitate dark brown (5YR 4/1)	coarse precipitate dark brown (5YR 3/1)

^{a} Turbidity (NTU). ^{b} Suspension color according to Munsell Color Chart (*30*); e.g., 7.5 YR 8/1 indicates 7.5 in hue, 8 in value, and 1 in chroma. ^{c} R = Al/catechin molar ratio.

Table 2. Absorbance of Solutions (<0.1 μM) at 472 and 664 nm

	absorbance			
	7 days		30 days	
reaction system	472 nm	665 nm	472 nm	665 nm
OH/Al = 3.0 (pH 7.0)				
AlCl$_3$ (blank)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
R = 0^a	0.20 ± 0.02	0.04 ± 0.01	0.50 ± 0.02	0.07 ± 0.01
R = 0.2	0.51 ± 0.02	0.09 ± 0.01	1.65 ± 0.02	0.18 ± 0.02
R = 0.4	0.95 ± 0.02	0.13 ± 0.01	1.69 ± 0.02	0.18 ± 0.01
R = 0.6	1.07 ± 0.02	0.14 ± 0.01	2.07 ± 0.04	0.19 ± 0.01
R = 0.8	1.10 ± 0.01	0.14 ± 0.01	2.33 ± 0.05	0.19 ± 0.01
R = 1.0	1.17 ± 0.02	0.15 ± 0.02	2.36 ± 0.01	0.20 ± 0.01
OH/Al = 2.5 (pH 5.5)				
AlCl$_3$ (blank)	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
R = 0	0.05 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	0.05 ± 0.01
R = 0.2	0.10 ± 0.02	0.05 ± 0.01	0.15 ± 0.02	0.05 ± 0.02
R = 0.4	0.20 ± 0.02	0.07 ± 0.01	0.28 ± 0.01	0.09 ± 0.01
R = 0.6	0.26 ± 0.01	0.08 ± 0.01	0.30 ± 0.04	0.09 ± 0.02
R = 0.8	0.41 ± 0.02	0.05 ± 0.01	0.56 ± 0.01	0.13 ± 0.01
R = 1.0	0.45 ± 0.03	0.07 ± 0.02	0.57 ± 0.01	0.14 ± 0.01

^{a} R = Al/catechin molar ratio.

Al in the precipitates generally increased with increasing Al/catechin molar ratios, and the contents of Al in the precipitates in the reaction systems at pH 5.5 were higher than those in the systems at pH 7.0 (**Table 5**). The contents of Al in the precipitates also increased with increasing initial Al concentration (**Figure 1**). Both the content of Al in the precipitates and the dry weight of the precipitates of the Al−catechin complex increased with increasing initial Al concentrations (**Table 4**). The amount of the precipitates and the initial Al concentration were highly correlated (r > 0.997). Little difference was observed between the percentage of organic C in the precipitates in the reaction system at pH 5.5 or 7.0.

Table 3. pH of Samples Aged for 7 and 30 Days

	suspension pH			
	OH/Al = 2.5 (pH 5.5)		OH/Al =3 .0 (pH 7.0)	
	7 days	30 days	7 days	30 days
AlCl$_3$ (blank)	5.33 ± 0.04	4.59 ± 0.02	6.23 ± 0.08	5.76 ± 0.12
R = 0^a	5.36 ± 0.02	5.18 ± 0.02	6.42 ± 0.03	6.05 ± 0.05
R = 0.2	5.36 ± 0.02	5.29 ± 0.02	6.79 ± 0.02	6.32 ± 0.04
R = 0.4	5.41 ± 0.01	5.37 ± 0.02	6.84 ± 0.02	6.53 ± 0.07
R = 0.6	5.40 ± 0.03	5.33 ± 0.03	6.87 ± 0.01	6.61 ± 0.04
R = 0.8	5.36 ± 0.02	5.32 ± 0.06	6.90 ± 0.01	6.69 ± 0.05
R = 1.0	5.36 ± 0.01	5.29 ± 0.06	6.93 ± 0.01	6.82 ± 0.09

^{a} R = Al/catechin molar ratio.

Table 4. Dry Weight of Precipitates (Milligrams) in the Reaction Systems Containing Catechin and/or Al^a

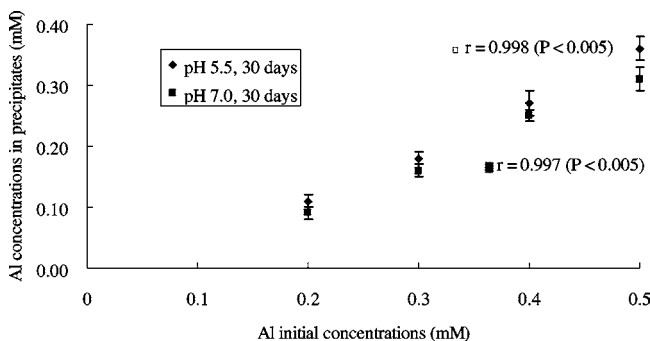|	OH/Al = 2.5 (pH 5.5)		OH/Al = 3.0 (pH 7.0)	
	7 days	30 days	7 days	30 days
AlCl$_3$ (blank)	0	0	0	0
R = 0^b	0	0	0	0
R = 0.2	trace	trace	trace	trace
R = 0.4	15 ± 1	17 ± 1	13 ± 2	16 ± 1
R = 0.6	31 ± 1	33 ± 2	26 ± 4	29 ± 2
R = 0.8	45 ± 1	46 ± 1	32 ± 2	40 ± 3
R = 1.0	49 ± 1	49 ± 2	41 ± 1	46 ± 2

^{a} The precipitates were obtained by centrifugation at 17700g for 10 min. ^{b} R = Al/catechin molar ratio.

Molar Ratios of Al/Catechin in Precipitates. The Al/catechin molar ratios in the precipitates increased with increasing initial Al/catechin molar ratios and were close to the initial Al/catechin molar ratios of the solutions (**Table 6** and **Figure 2**). The relationship between Al/catechin molar ratios

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Table 5. Al and Organic C Contents in Precipitates[a]

	Al content (g 100 g^{-1})		organic C (g 100 g^{-1})	
	7 days	30 days	7 days	30 days
OH/Al = 2.5 (pH 5.5)				
AlCl$_3$ (blank)	0	0	0	0
$R = 0$[b]	0	0	0	0
$R = 0.2$	trace	trace	trace	trace
$R = 0.4$	55.60 ± 0.40	55.80 ± 0.50	68.83 ± 0.2	69.02 ± 0.01
$R = 0.6$	58.80 ± 0.70	59.50 ± 0.70	66.88 ± 0.10	68.26 ± 0.04
$R = 0.8$	67.40 ± 0.40	67.90 ± 0.60	66.30 ± 0.03	66.55 ± 0.02
$R = 1.0$	70.70 ± 0.30	71.10 ± 1.00	63.20 ± 0.01	65.88 ± 0.16
OH/Al = 3.0 (pH 7.0)				
AlCl$_3$ (blank)	0	0	0	0
$R = 0$	0	0	0	0
$R = 0.2$	trace	trace	trace	trace
$R = 0.4$	47.30 ± 0.30	49.30 ± 0.80	68.36 ± 0.11	68.88 ± 0.03
$R = 0.6$	48.70 ± 0.40	53.20 ± 0.70	65.25 ± 0.02	65.71 ± 0.06
$R = 0.8$	56.60 ± 1.10	62.20 ± 1.00	63.50 ± 0.06	64.31 ± 0.09
$R = 1.0$	61.70 ± 1.00	65.20 ± 0.60	62.54 ± 0.03	63.21 ± 0.05

[a] The precipitates were obtained by centrifugation at 17700g for 10 min. [b] R = Al/catechin molar ratio.
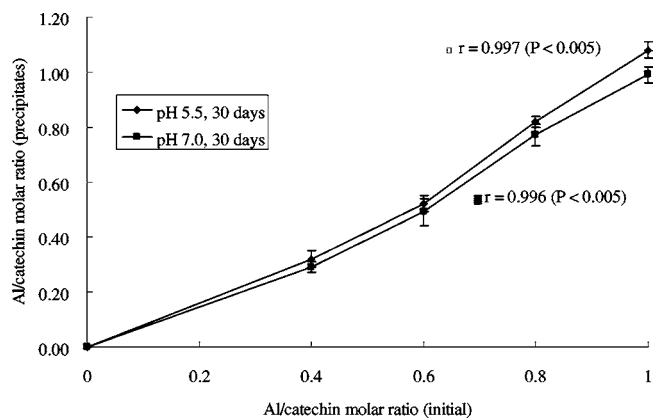

Figure 1. Relationship between initial Al concentration and Al concentration in the precipitates.

Table 6. Molar Ratios of Al to Catechin in Precipitates[a]

	Al/catechin molar ratio (in precipitates)			
initial molar ratio	OH/Al = 2.5 (pH 5.5)		OH/Al = 3.0 (pH 7.0)	
	7 days	30 days	7 days	30 days
AlCl$_3$ (blank)	0	0	0	0
$R = 0$[b]	0	0	0	0
$R = 0.2$	NA[c]	NA	NA	NA
$R = 0.4$	0.32 ± 0.03	0.32 ± 0.03	0.30 ± 0.04	0.31 ± 0.02
$R = 0.6$	0.53 ± 0.04	0.52 ± 0.03	0.45 ± 0.04	0.49 ± 0.05
$R = 0.8$	0.81 ± 0.04	0.82 ± 0.02	0.71 ± 0.03	0.77 ± 0.04
$R = 1.0$	1.12 ± 0.02	1.08 ± 0.03	0.99 ± 0.03	0.99 ± 0.03

[a] The precipitates were obtained by centrifugation at 17700g for 10 min. [b] R = Al/catechin molar ratio. [c] Not available. Due to trace amount of precipitates formed.

in the precipitates and initial Al/catechin molar ratios of the solutions shows high correlation ($r > 0.996$, $p < 0.05$). Therefore, we suggest that Al bonded with catechin, forming a 1:1 type complex.

XRD and TEM of Precipitates. The XRD pattern of catechin shows intense peaks at 1.65 and 0.40 nm and weak peaks at 1.12, 0.64, 0.54, 0.36, 0.33, and 0.31 nm [**Figure 3A(a)**].In the absence of Al at pH 5.5 (Al/catechin = 0), the reaction products even after 30 days of aging remained in crystalline phase, the same as catechin chemicals [**Figure 3A(a,b)**]. When Al was present (Al/catechin = 0.4, 0.6, 0.8, 1.0) in a system at the same pH, the precipitates were X-ray noncrystalline after 30 days of aging [**Figure 3A(c–f)**]. This is apparently due to Al complexation with catechin and the subsequent catechin polymerization. However, even in the

Figure 2. Relationship between initial Al/catechin molar ratio of the solution and Al/catechin molar ratio of the precipitates.
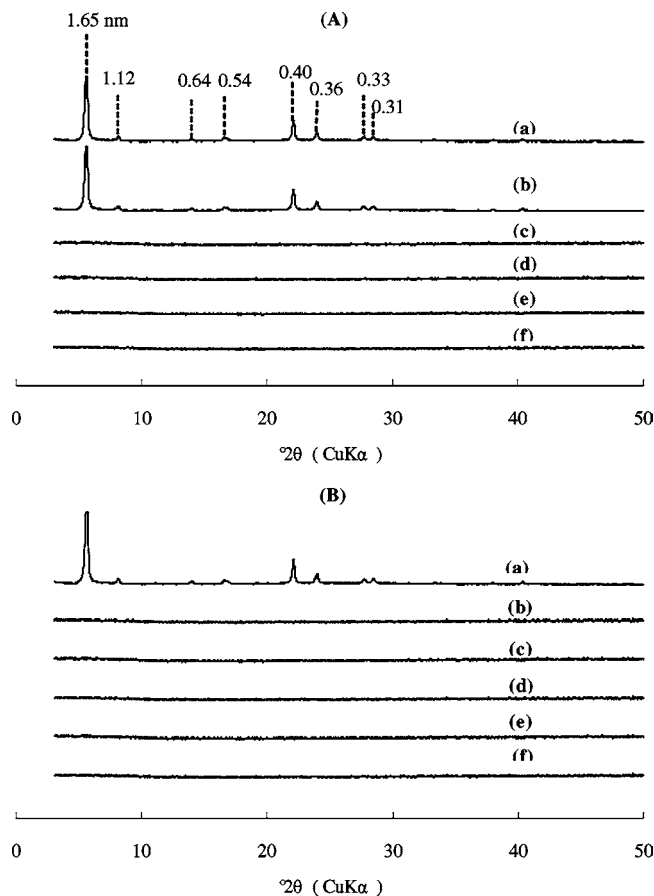
Figure 3. X-ray diffractograms of (**a**) catechin (chemicals), (**b**) Al/catechin = 0, (**c**) Al/catechin = 0.4, (**d**) Al/catechin = 0.6, (**e**) Al/catechin = 0.8, and (**f**) Al/catechin = 1.0. Samples **b**–**e** were prepared at (**A**) pH 5.5 and (**B**) pH 7.0 and aged for 30 days.

absence of Al at pH 7.0, the precipitates formed at the Al/catechin = 0 (only catechin) were X-ray noncrystalline precipitates [**Figure 3B(b)**], which is attributed to catechin oxidation and subsequent polymerization at pH 7.0. The XRD pattern of catechin shows the clear crystalline diffraction peaks at $2\theta = 5-30°$ (Al/catechin = 0, pH 5.5, 30 days) [**Figure 3A(b)**], but the crystalline X-ray diffraction peaks of catechin were absent (Al/catechin = 0, pH 7.0, 30 days) [**Figure 3B(b)**] (*10*), indicating that catechin polymerization was more intense at pH 7.0 than at pH 5.5.

The bright and dark field images of catechin (Al/catechin = 0, pH 5.5) show irregularly shaped particles (**Figure 4a,b**); the

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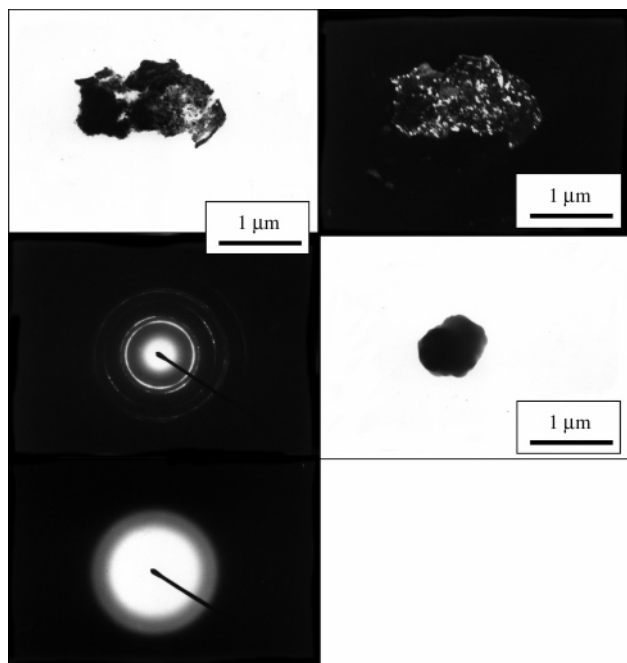
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Figure 4. Transmission electron micrographs of Al/catechin = 0 (pH 5.5) [(**a**) bright field image; (**b**) dark field images; (**c**) selected area electron diffraction pattern] and Al/catechin = 1.0 (pH 5.5) [(**d**) bright field image; (**e**) selected area electron diffraction pattern].

electron diffraction pattern of the sample shows that the material was crystalline (**Figure 4c**).The bright field image of the sample (Al/catechin molar ratio = 1.0, pH 5.5) shows that material was irregularly shaped (**Figure 4d**); no dark field image was obtained, and the material was noncrystalline to electron diffraction (**Figure 4e**). The TEM investigations show a trend similar to that of XRD analysis in that the reaction products of Al and catechin were noncrystalline, apparently through Al complexation with catechin and subsequent catalysis of Al in catechin polymerization.

ESR Spectra of Precipitates. The ESR spectra of the precipitates in the reaction systems at Al/catechin molar ratios of 0.8 and 1.0 show a single symmetrical line devoid of any fine splitting (**Figure 5**), which indicates the presence of free radicals, but at Al/catechin = 0 and for Al (blank) samples no free radical signals were found. The free radicals have *g* values of 2.0040−2.0042 with the line width of 3.334 G (**Figure 5**), indicative of semiquinones, the major free radicals present in the humic acid fractions of soil organic matter (*26*). The *g* value and the line width are close to the values reported for the humic acids produced from phenols (*27*).

¹³C CPMAS NMR Spectra of Precipitates. In the CPMAS spectra of catechin (Al/catechin = 0, pH 5.5, aged for 30 days), the peaks of chemical shift at 155.2 ppm (for C-5 and C-7), 154.0 (C-8a), 145.1 (C-4′), 144.1 (C-3′), 129.1 (C-1′), 125.0 (C-6′), 118.2 (C-5′), 116.0 (C-2′), 103.2 (C-4a), 101.3 (C-6), 97.4 (C-8), 81.2 (C-2), and 65.7 (C-3) and a broad band centered at 27.5 ppm (C-3) were observed (**Table 7**) (**Figure 6c**). The result of the control sample (only catechin) was close to the result of Antonio and Pedro (*28*) (**Figure 6b**). There were some differences in chemical shifts observed in the precipitates formed at Al/catechin = 0 and Al/catechin = 0.8, 1.0 samples (**Figure 6c−e**). At Al/catechin = 0.8 and 1.0, the chemical shifts of peaks at 154 ppm (C-8a), 145.1 ppm (C-4′), 144.1 ppm (C-3′), 125 ppm (C-6′), 118.2 ppm (C-5′), 103.2 ppm (C-4a) were absent (**Table 7** and **Figure 6a,d,e**). This indicates that the OH groups at C-3′ and C-4′ are lost due to Al complexation.
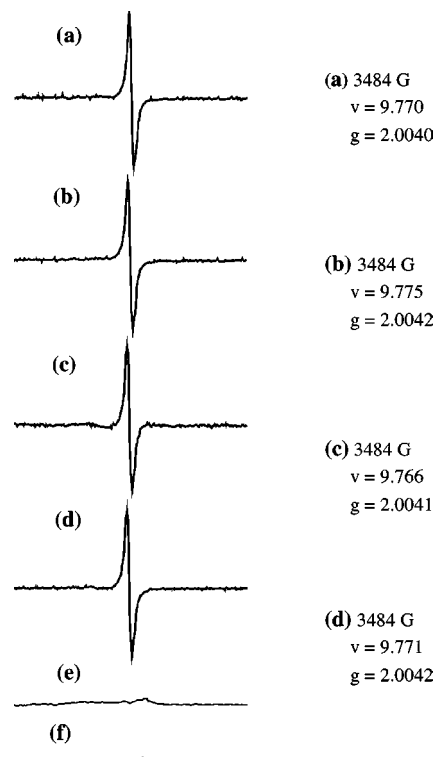

Figure 5. ESR spectra of the precipitates formed at pH 5.5: (**a**) Al/catechin = 0.8 and (**b**) Al/catechin = 1.0 aged for 7 days; (**c**) Al/catechin = 0.8, (**d**) Al/catechin = 1.0, (**e**) Al/catechin = 0, and (**f**) AlCl₃ (blank) aged for 30 days.

Table 7. Solid State ¹³C NMR Chemical Shifts of Precipitates (pH 5.5, Aged for 30 Days)

|	chemical shift (ppm)			
C-atom	Antonio and Pedro (*28*)	Al/catechin = 0	Al/catechin = 0.8	Al/catechin = 1.0
C-2	80.5	81.2	82.5	82.3
C-3	65.4	65.7	67.5	66.9
C-4	25.1	27.5	27.6	27.6
C-4a	103.4	103.2	–	–
C-6	100.9	101.3	99.8	100.1
C-8	97.0	97.4	96.8	97.6
C-5	155.6	155.2	155.1	155.2
C-7	155.6	155.2	155.1	155.2
C-8a	154.1	154	–[a]	–
C-1′	130.4	129.1	127.1	127.6
C-2′	115.9	116	114.2	114.6
C-3′	144.0	144.1	–	–
C-4′	144.8	145.1	–	–
C-5′	118.8	118.2	–	–
C-6′	125.4	125	–	–

[a] Peaks disappeared.

FT-IR Spectra of Precipitates. The wavenumber of FT-IR spectra (Al/catechin = 0, pH 5.5, aged for 30 days) at 827, 1030, 1122, 1146, 1290, 1470, 1522, and 1620 cm⁻¹ were assigned to C−H alkenes, −C−O alcohols, C−OH alcohols, −OH aromatic, C−O alcohols, C−H alkanes, C=C aromatic ring, and C=C alkenes (**Figure 7a,b**) (*29*). The absorption bands at 1030, 1122, 1146, and 1470 cm⁻¹ were absent in the presence of Al (Al/catechin = 0.4, 0.6, 0.8, and 1.0, pH 5.5, aged for 30 days) (**Figure 7c−f**). The other wavenumbers at 1290 and 1522 cm⁻¹ were also shifted to 1271 and 1502 cm⁻¹. The ¹³C NMR (**Figure 6**) and IR (**Figure 7**) studies indicate that Al complexed

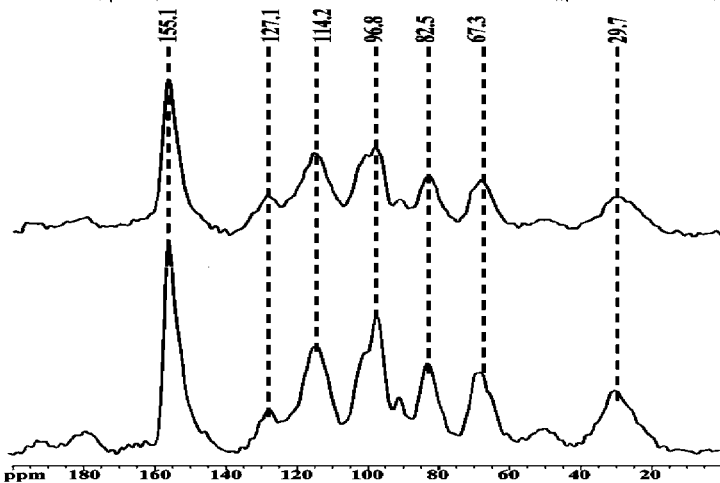Catechin Transformation

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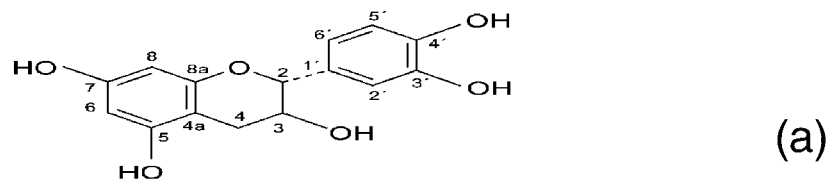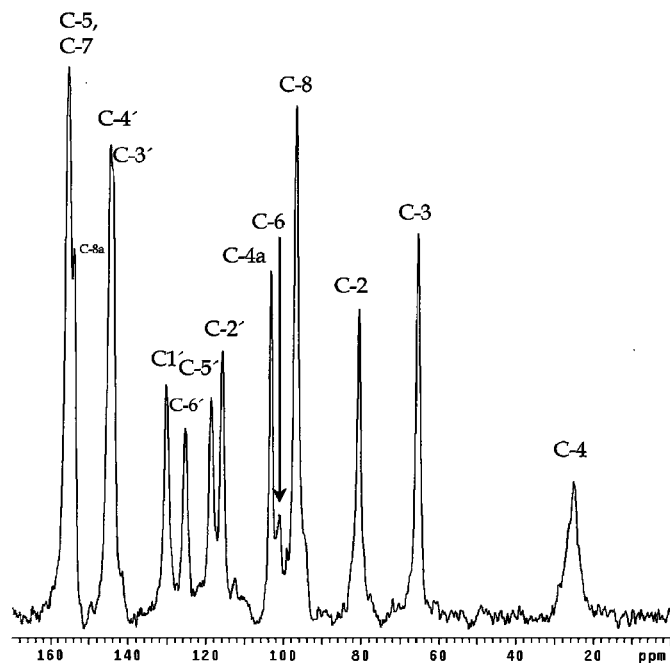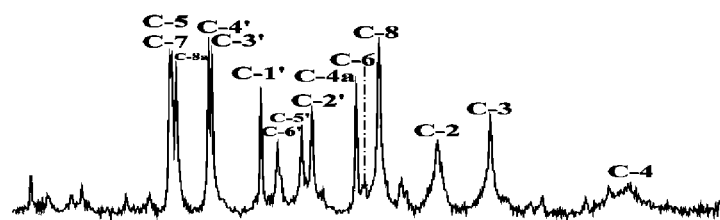
Figure 6. (**a**) Chemical structure and solid state ^{13}C NMR spectra of (**b**) catechin hydrate [adapted from Antonio and Pedro (*28*)], (**c**) Al/catechin = 0, (**d**) Al/catechin = 0.8, and (**e**) Al/catechin = 1.0. Samples **c**–**e** were prepared at pH 5.5 and aged for 30 days.

with catechin, causing the loss of some function groups (e.g., C-3′ OH and C-4′ OH) in IR and ^{13}C NMR spectra.

Conclusions. Catechin and Al are present in tea plants. However, to data, little is known of the chemistry of interaction of catechin biomolecules and Al ions. The visible absorption spectroscopic data show that Al ions substantially enhance the browning of (+)-catechin. The X-ray diffraction data show that crystalline catechin was transformed to X-ray noncrystalline precipitates upon interaction with Al ions. CPMAS ^{13}C NMR, FT-IR, and chemical analyses indicate that Al reacts with
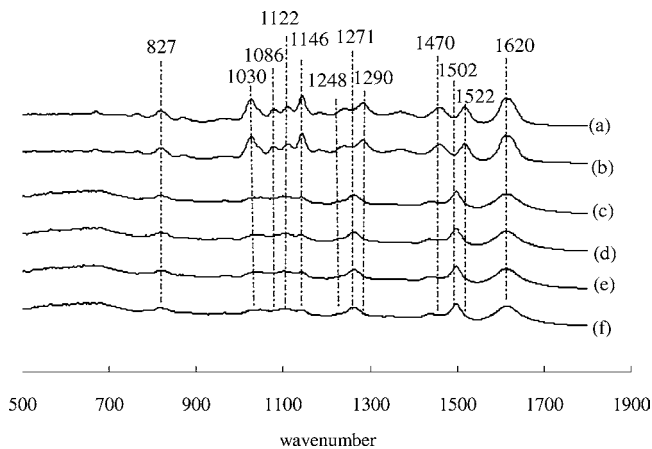
Figure 7. Infrared spectra of (**a**) catechin (chemicals); (**b**) Al/catechin = 0; (**c**) Al/catechin = 0.4; (**d**) Al/catechin = 0.6; (**e**) Al/catechin = 0.8; (**f**) Al/catechin = 1.0. Samples **b**–**e** were prepared at pH 5.5 and aged for 30 days.

phenolic O at C-3′ and C-4′ of catechin, resulting in the formation of a 1:1 type complex. The ESR spectra of the precipitates show the presence of semiquinone free radicals. The data obtained in the present study are of fundamental significance in advancing our understanding of the chemistry of catechin transformation as influenced by Al in the terrestrial ecosystem.

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