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Chemical stability and degradation mechanisms of ferulic acid (F.A) within various cosmetic formulations

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Synopsis

Ferulic acid (F.A) receives significant interest in the beauty industry with regard to its skin-whitening and anti-oxidant properties. However, its use in cosmetics is limited due to pH- and temperature-related instabilities. In this study, we investigated the stability of F.A in eight different prototype formulae. The results confirmed that in our conditions the stability of F.A is pH- and temperature-related. Additionally, the nature of the solvent dipropylene glycol (DPPG) showed a capacity to stabilize F.A. A series of experiments was further planned for studying the mechanism of degradation of F.A. In a prototype of a cosmetic medium, F.A degrades first through a decarboxylation step, leading to 4-hydroxy-3-methoxystyrene (PVG). Further, F.A and PVG are both involved in an additional reaction, resulting in the *trans*-conjugation dimer of PVG. The consequences of these results in formulating F.A are discussed.

INTRODUCTION

Traditional Chinese medicine (TCM) is probably the most ancient (*ca.* > 30 centuries) human medical or paramedical approach for preventing diseases, curing or sustaining body health. It comprises thousands of preparations from either vegetal, animal, or mineral extracts. These are blended by Chinese TCM pharmacists, according to "recipes" accumulated for centuries, written in secular texts. In brief, TCM can be regarded as the first human pharmacopeia. In practice, the TCM composition given to a patient by a pharmacist, for a given affliction, is rarely one extract but a mixture of different extracts that, according to TCM theories, work in a complementary or synergistic way. Most generally these are absorbed by an oral route. Beyond medical purposes, there is considerable public and scientific interest in the topical use of TCM ingredients as beauty magnifiers in the cosmetic field (1).

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Ferulic acid (F.A) is a phenolic phytochemical of the cinnamate family, present in a significant amount in vegetables, fruits, and beverages—e.g., in tomatoes, sweet corn, rice bran, coffee, peanuts, etc. It is also widely abundant in the TCM plant kingdom, such as in danggui (*Angelica sinensis*), chuanxiong (*Ligusticum wallichii*), muzei (Equisetum), and shengma (Rhizoma Cimicifugae), which are commonly used in TCM preparations. F.A is known as a major active ingredient of many TCMs and is therefore considered as a pivotal compound against a wide range of skin problems and is of interest to the cosmetics industry (2).

F.A is claimed to possess a wide spectrum of beneficial activities for skin care, i.e., alleviating cutaneous pigmentation (3), sun-induced darkening (4), inflammatory reactions (5), and the skin-aging process (6). Most of these properties seem linked to its strong antioxidative activity with regard to its phenolic nucleus and extended side-chain conjugation (Figure 1). A few works report that F.A can inhibit melanin production (7) and tyrosinase activity (8), making it a potential candidate for whitening products. In cosmetics formulations, F.A exhibits stability issues during long storage times, leading to color and/or odor changes in the final product. The work presented in this paper reports the stability criteria of F.A in various cosmetic formulations, together with its mechanism of degradation, in an attempt to define appropriate stabilizing conditions of F.A in cosmetic preparations.

MATERIALS AND METHODS

MATERIALS

Trans-ferulic acid (> 98% purity) was purchased from Ichimaru Pharcos Co., LTD (Lots No. U-808 and Q-728, Gifu, Japan) and used as received. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Water was supplied by a MilliQ water purifier system (Millipore, Bedford, MA). Sigma Chemical Co. (St. Louis, MO) provided 4-hydroxy-3-methoxystyrene (98%), and 1,3-bis (4-hyroxy-3-methoxyphenyl) -1-butene was purified by column chromatography in our laboratory to reach a purity >98%, as confirmed by HPLC. Sodium hydroxide, triethanolamine (TEA), and dipropylene glycol (DPPG) were obtained from industrial suppliers to the cosmetic industry, all of high purity grade. Silica gel-coated TLC plates (silica gel 60, 0.10–0.20-mm thickness) and 100–200-mesh silica gel for column chromatography was purchased from Wusi Chemical Company. All solvents and solutions for HPLC analysis were filtered, prior to use, through a Millipore filter (pore size 0.45 μm).

METHODS

Preparation of F.A in existing and modified formulae. Eight representative formulae were defined in formulating 1% F.A (w/w) at different pH levels. Cream-based formulae were pH

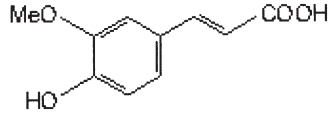


Figure 1. Molecular structure of *trans*-ferulic acid.

Table I
Stability Results of Creams 1–3 and Serums 4–8 under All Conditions
(a): Stability of Cream 1-3 @ RT/dark[8]

Cream			AspectColorOdor	⊿pH[2]	F.A%[3] (RT/dark)
1[1]	pH	blank	No change	—	—
		4.5	No change	—	0.942[4]
		5.5	No change	—	0.949[4]
		6.5	No change	—	0.916[4]
2[1]	pH	blank	No change	—	—
		4.5	No change	—	0.991[4]
		5.5	No change	—	0.971[4]
		6.5	Impossible to formulate	—	—
3	pH	blank	No change	—	—
		5.1[6]	Slight O change	+0.10	0.963[4]
		5.5	Slight O change	−0.05	0.981[4]
		6.5	Diphase, C & O change	+0.23	0.972[4]

See p. 488 for footnotes relating to all parts of Table I.

(b): Stability of Creams 1-3 @ RT/light[8]

Cream			AspectColorOdor
1[1]	pH	blank	No change
		4.5	Slightly yellowish
		5.5	Slightly yellowish
		6.5	Slightly yellowish
2[1]	pH	blank	No change
		4.5	No change
		5.5	Slight C & O change
		6.5	Impossible to formulate
3	pH	blank	No change
		5.1[6]	Slight C & O change
		5.5	Slight C & O change
		6.5	Slight C & O change

(c): Stability of Creams 1-3 @ 4°C/dark[8]

Cream			AspectColorOdor	F.A%[3] (4°C/dark)
1[1]	pH	blank	No change	—
		4.5	No change	0.990[4]
		5.5	No change	0.990[4]
		6.5	No change	0.991[4]
2[1]	pH	blank	No change	—
		4.5	No change	0.991[4]
		5.5	No change	0.990[4]
		6.5	Impossible to formulate	—
3	pH	blank	No change	—
		5.1[6]	No change	0.991[4]
		5.5	No change	0.990[4]
		6.5	No change	0.991[4]

(d): Stability of Creams 1-3 @ 37°C/dark[8]

Cream			AspectColorOdor
1 [1]	pH	blank	Thicker, but acceptable
		4.5	Thicker, C & O change
		5.5	Severe C change
		6.5	Severe C & O change
2 [1]	pH	blank	No change
		4.5	Slight C & O change
		5.5	Severe C change
		6.5	Impossible to formulate
3	pH	blank	Thicker, but acceptable
		5.1[6]	C & O change
		5.5	C & O change
		6.5	Diphase, C & O change

(e): Stability of Creams 1-3 @ 45°C/dark[8]

Cream			AspectColorOdor	⊿pH[2]	F.A%[3] (45°C/dark)
1 [1]	pH	blank	Thicker, but acceptable	—	—
		4.5	Thicker, C & O change	—	0.534[4]
		5.5	Thicker, Severe C change	—	0.353[4]
		6.5	Thicker, Severe C change	—	0.215[4]
2 [1]	pH	blank	No change	—	—
		4.5	C & O change	—	0.583[4]
		5.5	Diphase, severe C change	—	0.365[4]
		6.5	Impossible to formulate	—	—
3	pH	blank	Thicker, but acceptable	0	—
		5.1[6]	Severe C & O change	−0.1	0.670[4]
		5.5	Severe C & O change	+1.0	0.581[4]
		6.5	Diphase, severe C & O change	+1.2	0.404[4]

(a-1): Stability of Serums 4-8 @ RT/dark[8]

Serum			AspectColorOdor	⊿pH[2]	F.A%[3] (RT/dark)
4	pH	blank	No change	−0.1	—
		5.0	No change	−0.0	0.979[5]
		6.0	No change	+0.3	0.951[5]
5	pH	blank	No change	+0.1	—
		5.0	Impossible to formulate	—	N/A
		6.2[7]	No change	+0.2	0.969[4]
6	pH	blank	No change	+0.0	—
		5.0	Impossible to formulate	—	N/A
		6.0	No change	+0.0	0.981[4]
7 [1]	pH	blank	No change	—	—
		5.0	Crystal↓	—	0.962[5]
		6.0	No change	—	0.945[5]
8	pH	blank	No change	−0.1	—
		5.0	Crystal↓	—	N/A
		6.0	No change	+0.1	0.972[5]

(b-1): Stability of Serums 4-8 @ RT/light[8]

Serum			AspectColorOdor
4		blank	No change
	pH	5.0	No change
		6.0	Slight C change
5		blank	No change
	pH	5.0	Impossible to formulate
		6.2[7]	No change
6		blank	No change
	pH	5.0	Impossible to formulate
		6.0	No change
7[1]		blank	No change
	pH	5.0	Crystal↓
		6.0	No change
8		blank	No change
	pH	5.0	Crystal↓
		6.0	Slight C change

(c-1): Stability of Serums 4-8 @ 4°C/dark[8]

Serum			AspectColorOdor	F.A%[3] (4°C/dark)
4		blank	No change	—
	pH	5.0	No change	0.989[5]
		6.0	No change	0.990[5]
5		blank	No change	—
	pH	5.0	Impossible to formulate	N/A
		6.2[7]	No change	0.990[4]
6		blank	No change	—
	pH	5.0	Impossible to formulate	N/A
		6.0	Diphase	0.990[4]
7[1]		blank	No change	—
	pH	5.0	Crystal↓	0.991[5]
		6.0	No change	0.991[5]
8		blank	No change	—
	pH	5.0	Crystal↓	N/A
		6.0	No change	0.991[5]

(d-1): Stability of Serums 4-8 @ 37°C/dark[8]

Serum			AspectColorOdor
4	pH	blank	No change
		5.0	No change
		6.0	C change
5	pH	blank	No change
		5.0	Impossible to formulate
		6.2[7]	Severe C change
6	pH	blank	No change
		5.0	Impossible to formulate
		6.0	Diphase, severe C change
7[1]	pH	blank	No change
		5.0	Severe C & O change
		6.0	Severe C & O change
8	pH	blank	No change
		5.0	Severe C & O change
		6.0	Severe C change

(e-1): Stability of Serums 4-8 @ 45°C/dark[8]

Serum			AspectColorOdor	⊿pH[2]	F.A%[3] (RT/dark)
4	pH	blank	No change	−0.4	—
		5.0	Slight C & O change	−0.3	0.713[5]
		6.0	Severe C change	+1.1	0.476[5]
5	pH	blank	No change	−0.4	—
		5.0	Impossible to formulate	—	N/A
		6.2[7]	Severe C change	+1.4	0.531[4]
6	pH	blank	No change	+0.0	—
		5.0	Impossible to formulate	—	N/A
		6.0	Diphase, severe C & O change	+0.8	0.689[4]
7[1]	pH	blank	No change	—	—
		5.0	Severe C & O change	—	0.679[5]
		6.0	Severe C & O change	—	0.624[5]
8	pH	blank	No change	0	—
		5.0	Severe C & O change	—	N/A
		6.0	Severe C change	+1.4	0.670[5]

[1] In W/O and W/Si formulae, pH can't be detected, so pH here refers only to that of water phase.
[2] ⊿pH here refers to the pH difference between the initial one and the one incubated under RT/45°C after 2 months.
[3] F.A% here refers to the remaining percentage of FA from initial 1%. For example, 0.720 represents that after 2 months' storage. F.A decreased from 1.0% to 0.720% in formula.
[4] Tested after 42 days' storage.
[5] Tested after 60 days' storage.
[6] Below this pH, 1% of F.A can not be totally dissolved in the water phase of cream 3.
[7] Below this pH, 1% of F.A can not be totally dissolved in the water phase of serum 5.
[8] F.A retention percentage @ RT/light and 37°C/dark was not detected.

Table II
Stability Result of Modified Formula—Cream 9 and Serum 10
(a): Stability @ RT/dark[8]

Fla	pH	ACO	⊿pH[1]	F.A%[2] (RT/dark)
Cream 9	4.7	No change	−0.05	0.949
Serum 10	4.5	No change	+0.04	0.969

(b): Stability @ RT/light[8]

Fla	pH	ACO	⊿pH[1]
Cream 9	4.7	No change	—
Serum 10	4.5	No change	—

(c): Stability @ 4°C/dark[8]

Fla	pH	ACO	⊿pH[1]	F.A%[2] (4°C/dark)
Cream 9	4.7	No change	—	1.000
Serum 10	4.5	No change	—	0.990

(d): Stability @ 37°C/dark[8]

Fla	pH	ACO	⊿pH[1]
Cream 9	4.7	No change	—
Serum 10	4.5	No change	—

(e): Stability @ 45°C/dark[8]

Fla	pH	ACO	⊿pH[1]	F.A%[2] (45°C/dark)
Cream 9	4.7	No change	+0.07	0.806
Serum 10	4.5	Very slight C change	+0.15	0.786

[1] ⊿pH here refers to the pH difference between the initial one and the one incubated under RT/45°C after 2 months.
[2] F.A% here refers to the remaining percentage of FA from the initial 1%. Each result was tested after 2 months' incubation. For example, 0.806 represents that after 2 months' storage, F.A decreased from 1.0% to 0.806% in the formula.
[3] F.A retention percentage @ RT/light and 37°C/dark was not detected.

adjusted to 4.5, 5.5, and 6.5 and to pH 5.0 and 6.0 for serum-based ones. One cream and one serum were chosen from the above for modification purposes, replacing the whole-water phase by 30% DPPG and adjusting the percentage of deionized water.

Stability tests of F.A formulae. The F.A-containing formulae were prepared in transparent bottles and placed at different temperatures (RT, 4°C, 37°C, and 45°C in dark conditions) or on a shelf (stored under uncontrolled room temperature and exposed to ambient light) for two months. During storage time, the stability of the formulations were assessed through their changes in aspect, color, and odor status (A.C.O.), and pH/viscosity

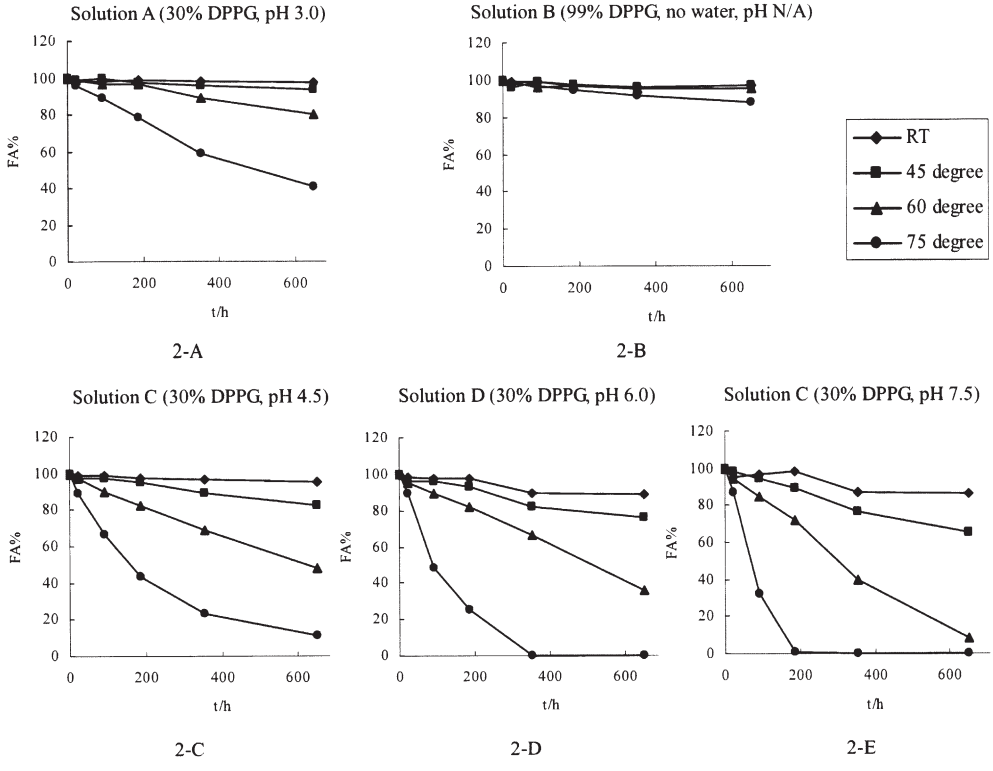
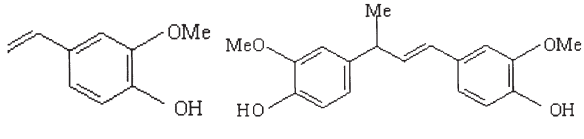


Figure 2. Effect of temperature and time of incubation on the degradation of F.A in solutions A–E.

Table III
Composition of Solutions A–E

	Weight percentage				
Solution	F.A	DPPG	H_2O	Base	pH
A	1%	30%	69%	—	3.0
B	1%	99%	—	—	—
C	1%	30%	Up to 100%	NaOH	4.5
D	1%	30%	Up to 100%	NaOH	6.0
E	1%	30%	Up to 100%	NaOH	7.5

The compositions are expressed as weight percentage.



Compound 1: PVG Compound 2: *Trans*-Dimer of PVG

Figure 3. Structure of two F.A degradation products—PVG and its *trans*-dimer.

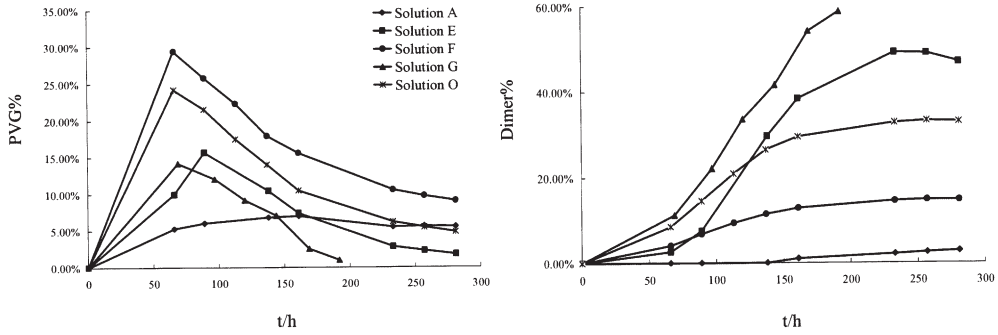


Figure 4. Effect of NaOH, KOH, TEA, and TEA with salt on the accumulation of PVG and its dimer during the degradation of F.A. We calculated the conversion rate of PVG and that of its dimer by tracing the benzene rings in the solutions, which means that a complete conversion of one molecule of F.A could theoretically produce one molecule of PVG or 0.5 molecule of the dimer. Thus, 1% (w/w) of F.A could transform up to 0.77% (w/w) of PVG or 0.77% (w/w) of its dimer. The PVG% and dimer% here are calculated according to this theoretical yield.

Table IV
Composition of Solutions A, E, F, G, and O

	Weight percentage					
Solution	F.A	DPPG	H_2O	Base	pH	Additional
A	1%	30%	69%	—	3.0	—
E	1%	30%	Up to 100%	NaOH	7.5	—
F	1%	30%	Up to 100%	TEA	7.5	—
G	1%	30%	Up to 100%	KOH	7.5	—
O	1%	30%	Up to 100%	TEA	7.5	5.85% NaCl

The compositions are expressed as weight percentage.

and F.A retention rate, using a standard HPLC technique. The latter was comprised of an Agilent™ 1200 Series liquid chromatograph, using a delivery pump with an online degasser, an injector with a variable loop, and a photodiode array detector through an X-Terra RP18 (3.9 mm × 150 mm, 5 μm; Waters) column. The equipment and processes were automatically controlled by "Chemstation revision A.08.03" software (Agilent Technology). The elution was carried out through solution A (0.1% TFA w/w in an aqueous solution of methanol 60:40 v/v, respectively) for 15 minutes. The chromatographic process used a flow rate of 1.0 ml·min^{-1}, a column temperature of 30°C, and an injection volume of 10 μl, with a detection wavelength fixed at 323 nm.

Sample preparation in simplified systems. A series of model solutions of F.A at different pH levels was prepared. One hundred grams of the two simplified solutions contained (w/w) the following components: (i) solution A—F.A 1.0 g; DPPG, 30.0 g; water, 69.0 g; and (ii) solution B—F.A, 1.0 g; DPPG, 99.0 g. The former basal solution was modified to achieve new systems by adjusting the pH to 4.5, 6.0, and 7.5 with NaOH (solutions C, D, and E; see Table IV). Further modifications were carried out as follows: TEA and KOH were used to neutralize the basal solution to pH 7.5 (solutions F and G; see Table IV). A full replacement of F.A by PVG (1%, w/w) was performed both in the basal solution and

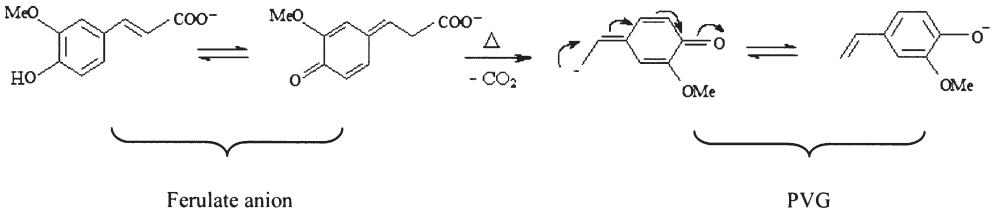


Figure 5. Proposed mechanism for F.A degradation—Step one: decarboxylation.

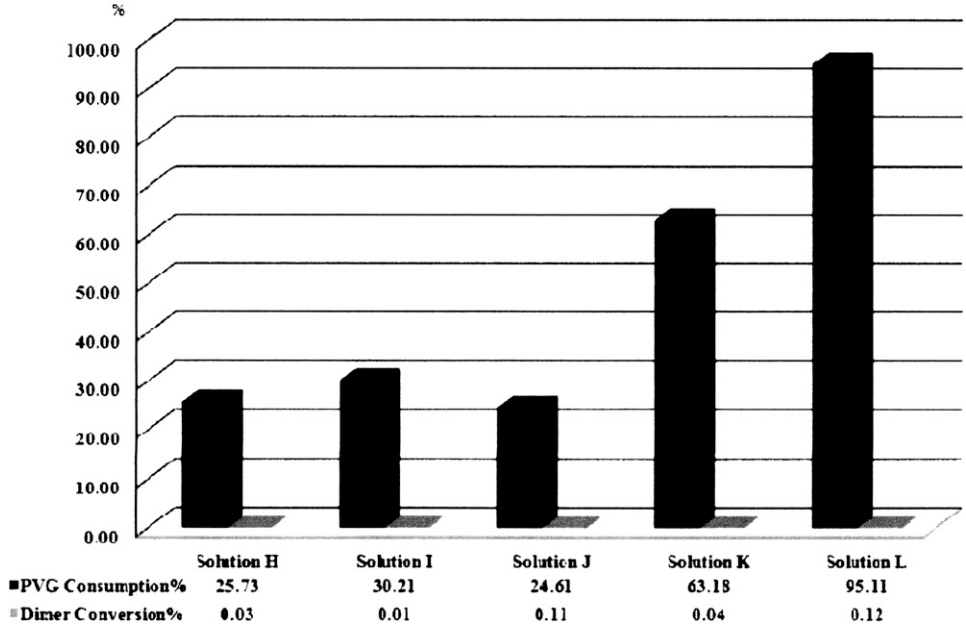


Figure 6. Accumulation and/or consumption of PVG and its dimer in tested solutions. Solutions were incubated at 75° for seven days. The PVG consumption% was calculated according to its initial concentration (1%, w/w), and the dimer conversion% here was calculated in the same way as in Figure 4.

Table V
Composition of Solutions H–L

	Weight percentage				
Solution	PVG	DPPG	H_2O	Base	pH
H	1%	30%	Up to 100%	—	4.65
I	1%	30%	Up to 100%	NaOH	7.5
J	1%	30%	Up to 100%	TEA	7.5
K	1%	30%	Up to 100%	NaOH	9.0
L	1%	30%	Up to 100%	TEA	9.0

The compositions are expressed as weight percentage.

the ones at pH 7.5, neutralized by NaOH and TEA (solutions H, I, and J; see Table V). Solution H was respectively neutralized to pH 9.0 by NaOH and TEA (solutions K and L; see Table V). PVG (1%, w/w) was added to the basal solution (solution M; see Table VI) and to solution E (solution N; see Table VI). NaCl (5.85%, w/w) was added to the solution and further neutralized to pH 7.5 by TEA (solution O; see Table VII). Addition of Vitamin C (0.2%, w/w) was added to the solution, together with a sweeping oxygen flow (solution P; see Table VII). A nitrogen (N_2) atmosphere was obtained through nitrogen

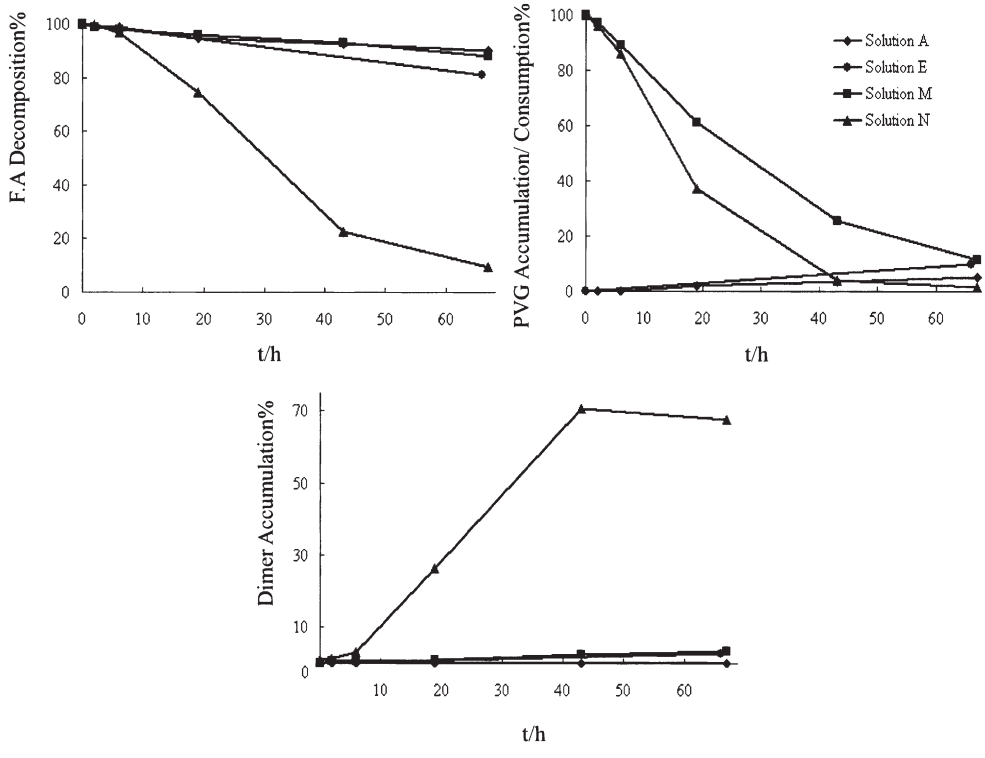


Figure 7. Accumulation and/or consumption of PVG and its dimer in tested solutions. Solutions were incubated at 75° for three days. The PVG consumption% was calculated according to its initial concentration (1%, w/w), and the PVG and dimer accumulation% here were calculated in the same way as in Figure 4.

Table VI
Composition of Solutions A, E, M, and T

	Weight percentage					
Solution	F.A	DPPG	PVG	H_2O	Base	pH
A	1%	30%	—	69%	—	3.0
E	1%	30%	—	Up to 100%	NaOH	7.5
M	1%	30%	1%	68%	—	4.2
N	1%	30%	1%	Up to 100%	NaOH	7.5

The compositions are expressed as weight percentage.

flow within solutions F and L (solutions Q and R; see Table VII). An oxygen atmosphere was obtained by oxygen flow within solution F (solution S; see Table VII).

The first five solutions (solutions A–E) were incubated at either RT, 45°C, 60°C and 75°C, and the concentration of F.A in all samples was determined by HPLC (conditions were the same as above) at various time periods up to four weeks. Other samples of the 19 solutions were incubated at 75°C for speeding up the degradation processes. Hence, concentration of F.A and its derivatives (see below) were analyzed by HPLC at different time periods under the following technical conditions: Hypersil BDS-C18 (4.6 mm × 250 mm, 5μm; Dalian Elite); mobile phase, 0.167 M acetic acid (solvent A) and methanol (solvent B); gradient applied, increasing solvent B from 40% to 65% within 3 min, holding 8 min; flow rate: 1.2 ml·min^{-1}, then increasing solvent B from 65% to 100% within 1 min, holding 5 min; flow rate: 2.0 ml·min^{-1}, decreasing solvent B from 100% to 40% within 1 min, holding 5 min; flow rate: 1.2 ml·min^{-1}, end; column temperature, 30°C; UV detector fixed at 254 nm; injection volume, 10 μl.

Characterization of products from F.A degradation. A moderate amount of oil-like precipitation was observed during the incubation period. These precipitates were separated by flash column chromatography, eluted with hexane and ethyl acetate mixture, starting with 100% hexane, with a later decrease in the ratio of hexane/ethyl acetate (v/v) to 80:20, 75:25, 67:33, to 100% ethyl acetate. The separated and purified fractions were then further identified by LC-MS and NMR analysis and kept as standard samples.

RESULTS AND DISCUSSION

STABILITY OF F.A IN DIFFERENT COSMETICS FORMULAE

In our work the eight representative formulae were specifically designed through the requirements of our internal sensory evaluation panel (textures close to those of a whitening product). We evaluated different types of formulations for enlarging the scope of factors prone to influence the stability of F.A. The compositions of these eight formulae are shown in Appendix I and Appendix II. The eight formulae were prepared without F.A, as blanks. Table I summarizes changes in ACO status, ΔpH, and % of F.A.

As shown in Table I, none of the tested formulae appeared stable compared to their respective blanks. Most formulae turned yellowish, together with odor changes, illustrating the two major stability problems encountered in F.A-based formulae. A strong increase in pH and a significant F.A degradation were observed in most cases.

Two formulae, cream 3 (pH 4.5) and serum 4 (pH 5.0) appeared somewhat "stable". These were selected for further modification. Two new formulae, cream 9 and serum 10, were created (compositions are in Appendix III). Their stability results shown in Table II

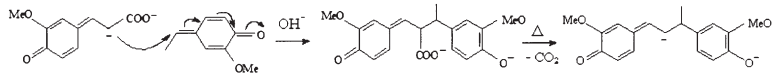


Figure 8. Proposed mechanism for the formation of dimer from F.A in tested solutions—Step two: additional reaction.

indicate that the stability of these F.A-based formulae clearly improved. Yellowing and odor issues were not observed, whereas the remaining fraction of F.A reached 81% in cream 9 and 79% in serum 10 after two months at 45°C.

These results suggest that pH, temperature, and DPPG content appear to be possible factors affecting the stability of F.A in formulae. To further evaluate their influence in the degradation process of F.A, the stability testings of this compound were then performed in more simplified systems, using water and/or DPPG as solvents.

F.A'S STABILITY AND DEGRADATION PRODUCTS IN SIMPLIFIED SYSTEMS

Effect of temperature, pH, and solvents on F.A degradation. The simplified systems were comprised of a series of aqueous dipropyleneglycol solutions (69/30, w/w) at pH 3.0 (F.A as free acid), 4.5, 6.0, and 7.5 (where F.A is completely in the form of sodium ferulate), and another in a 100% DPPG solution (F.A un-neutralized). These solutions were incubated at different temperatures, and their respective contents in F.A were further analyzed by HP.LC. Results, shown in Figure 2 and Table III, confirm that the instability of F.A is pH, temperature, and solvent dependent. Degradation of F.A increases with increased temperature and pH. The degradation rates show strong differences between RT, 45°, 60°, and 75°. With the exception of solution B, the rates at 75°C appeared significantly higher than those found at lower temperatures. The level of pH affects the degradation of F.A in a similar way, suggesting that F.A is more stable within a low-pH environment. The solvent DPPG showed a significant effect in slowing down the process of F.A degradation. A comparison of the results in Figure 2A and 2B allows one to conclude that F.A degrades about five times faster in an aqueous DPPG solution compared to 99% DPPG solution.

Characterization of F.A degradation products. The HPLC chromatograms and mass spectra of different F.A solutions, under various incubation conditions (different pH and/or temperatures for up to 60 days), showed that the F.A degradation products are similar (Appendix IV, Figure 11/Table XII). The peak retention time of F.A, in all cases, was 4.0 min.

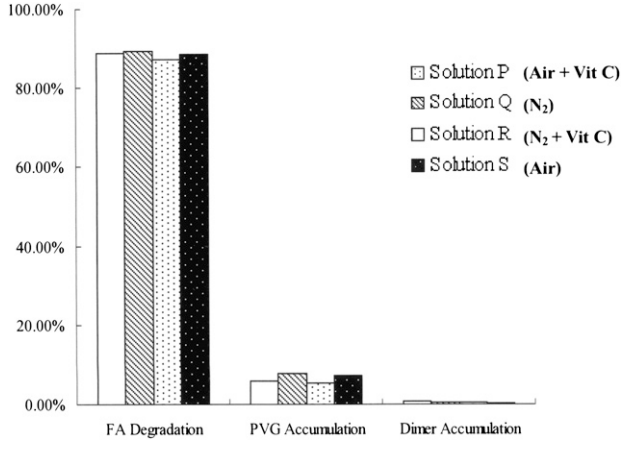


Figure 9. Effects of vitamin C and N_2/air atmosphere on F.A degradation and PVG and its dimer's formation in tested solutions. Solutions were incubated at 75° for five days. The PVG and dimer accumulation% here were calculated in the same way as in Figure 4.

Table VII
Composition of Solutions P–S

	Weight percentage					
Solution	F.A	DPPG	H_2O	Base	pH	Additional
P	1%	30%	Up to 100%	TEA	7.5	Sweep with air + 0.2% Vit. C
Q	1%	30%	Up to 100%	TEA	7.5	Sweep with N_2
R	1%	30%	Up to 100%	TEA	7.5	Sweep with N_2 + 0.2% Vit. C
S	1%	30%	Up to 100%	TEA	7.5	Sweep with air

The compositions are expressed as weight percentage.

Basically, two peaks represent two major F.A degradation products. Accordingly, we further processed the residue that was obtained from solution E, incubating at 75° for five days and 12 days, then submitting to preparative HPLC according to the following procedure.

The solutions were evaporated to oil-like residues and further column-chromatographed over silica gel (9). The eluates showed two major degradation products, 4-hydroxy-3-methoxystyrene (also named as p-vinylguaiacol, PVG) and 1,3-bis (4-hyroxy-3-methoxyphenyl)-l-butene (dimer of PVG) (refer to chemical structures in Figure 3). LCMS data exactly matched those of peaks at Rt = 7.3 and 10.0 min, respectively. Other trace components cannot be separated quantitatively.

Further NMR and IR tests were carried out for confirming the structure of these degradation products. Detailed MS and NMR data are listed in Appendix V and are in agreement with the study of Fiddler and Parker (10).

Kinetics of formation and behaviors of PVG and its dimer during F.A degradation. As F.A was the only "source" of a benzene ring in our experimental conditions, the isolation of these two products provided evidence that PVG and its dimer originate from F.A.Their respective kinetics of formation under different test conditions were further analyzed, and results are shown in Figure 4 and Table IV. With the exception of the un-neutralized solution A*, the kinetics of PVG formation showed similar shapes, perfectly fitting with those of the intermediates. The contents of PVG peaked at approximately 80 hours of incubation, progressively decreasing thereafter. In contrast, the conversions of the dimer were all kept at low levels during the first 80 hours. This fact suggests that the degradation of F.A is a complex reaction, implying a decarboxylation process as a first step, leading to PVG (Figure 5) (11).

In addition, almost no dimer was detected in the incubated solutions H–L, suggesting that PVG alone could not produce a dimer (Figure 6, Table V). As for the solutions containing additional PVG (solutions M and N), the induction time of dimer accumulation appeared shortened in solution N, mostly and to some extent in solution M (Figure 7, Table VI).

From the results above we assume that the accumulation of the dimer reflects the consumption of PVG, whereas F.A could be converted to dimer via an indirect route with

*In solution A, F.A was much more stable than in the others and took much longer time to degrade completely; and so through our study time, only a small amount of F.A was degraded in this system, and we were not able to get enough data to complete its accumulation curve.

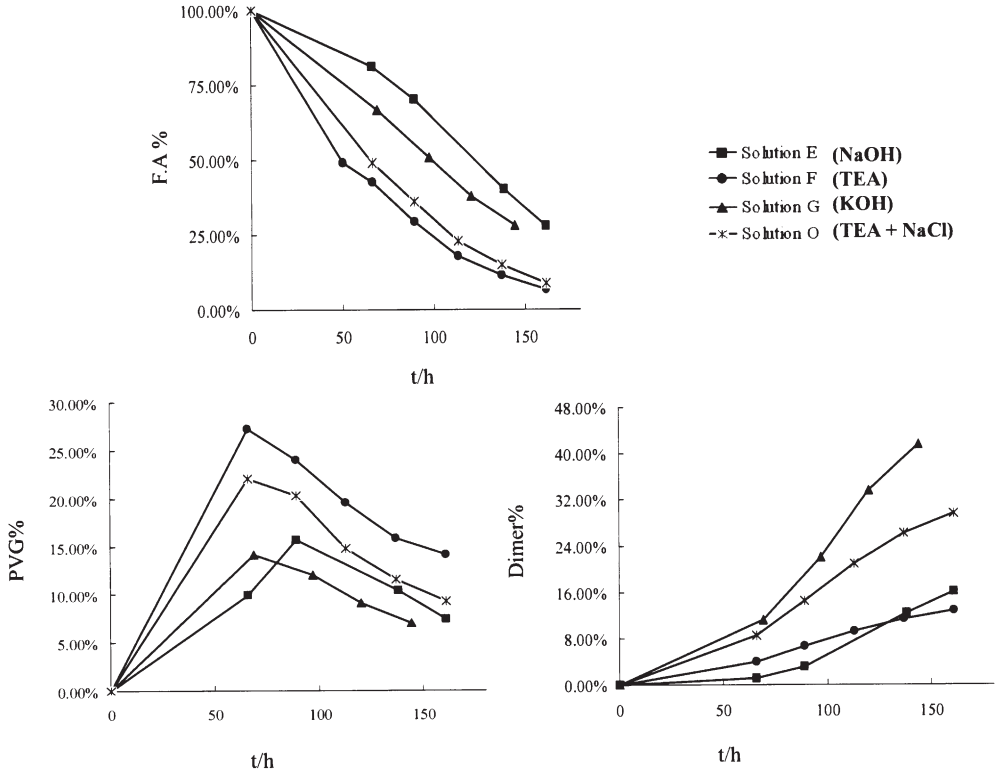


Figure 10. Effects of metallic cations on F.A degradation and PVG and its dimer's formation in tested solutions. Solutions were incubated at 75° for seven days. The PVG and dimer accumulation% here were calculated in the same way as in Figure 4.

PVG formation. These transformations, favored by the presence of OH^- ions (ferulate anion conjugations), seem to follow the proposed pathways shown in Figure 8.

As for the use of F.A in skincare products when F.A is likely to contact with bare skin, one should question the interaction of F.A when exposed to "real life" conditions of human stratum corneum and living epidermis. Simulation of F.A-based formulae with reconstructed epidermis as a model would be an interesting experimental approach. In fact, some microbial species, including bacteria, yeasts, and filamentous fungi (12–14) have been reported as being capable of degrading F.A. Microorganisms may possibly decarboxylate

Table VIII
Composition of Solutions E–G and O

	Weight percentage					
Solution	F.A	DPPG	H_2O	Base	pH	Additional
E	1%	30%	Up to 100%	NaOH	7.5	—
F	1%	30%	Up to 100%	TEA	7.5	—
G	1%	30%	Up to 100%	KOH	7.5	—
O	1%	30%	Up to 100%	TEA	7.5	5.85% NaCl

The compositions are expressed as weight percentage.

in situ on skin, or degrade it through other pathways. The biological roles of the F.A-derived products are still unclear, since both PVG and its dimer are unstable. Additional work is required to elucidate the respective roles of F.A, PVG, and its dimer in the process of skin depigmentation.

Effect of N_2 atmosphere, vitamin C, and metallic cations on F.A degradation. The effect of N_2 atmosphere and vitamin C on the formation of PVG and its dimer during the degradation of F.A is shown in Figure 9 and Table VII. The kinetics of degradation of F.A were slightly reduced under N_2 atmosphere, albeit not significantly, compared to normal atmosphere; N_2 did not inhibit the formation of PVG or dimer, suggesting that F.A does not degrade through an O_2^- involved radical mechanism.

The effect of metallic cations (Na^+/K^+) is shown in Figure 10 and Table VIII. The presence of Na^+/K^+ was especially relevant to the degradation of F.A to PVG and its dimer. Na^+/K^+, as expected, enhanced the formation of the dimer. On the other hand, these cations significantly and surprisingly reduced both the general content level and the peak concentration of PVG. The influence of K^+ appeared stronger than that of Na^+. These findings suggest that the metallic cations may catalyze and orientate the degradation of F.A towards the dimer rather than other degradation products. Due to the effect of Na^+/K^+, the balance between PVG formation and conversion to its dimer (or trimer) form may be shifted to the latter possibility, explaining the reduction in the accumulation of PVG. The presence of vitamin C clearly reduced the yellowing of the solutions at early incubation times. This had no significant effect on the formations of PVG or its dimer.

Despite the N_2 atmosphere and the presence of the well-known free-radical chain scavenger vitamin C, no significant effect on the inhibition of F.A degradation and PVG formation was observed. Metallic cations showed a remarkable catalytic effect on the formation of the dimer, and the K^+ of lower electronegativity appears much more efficient in the catalysis than does the Na^+. The environmental solvent of F.A clearly affects the degradation of F.A. Water favors the progression while DPPG slows it down. The presence of metallic cations, leading to inhibit the accumulation of PVG, suggests that PVG was mainly formed through an ionic mechanism under our experimental conditions. From all these results, it appears that for ensuring long-term stability of F.A, an acid and high-DPPG environment, together with the absence of metallic cations, offers a much better practical approach in F.A-based cosmetic formulations.

CONCLUSIONS

Our results suggest that F.A is a thermally unstable substance, the stability of which is affected by pH and certain solvents. A low pH, a low temperature, and a high percentage of DPPG appear to be efficient factors in ensuring a better chemical stability of F.A in a cosmetic medium. In addition, our analytical data suggest that PVG and its *trans*-conjugation dimer are the major degradation products of F.A, probably through an ionic mechanism rather than an O_2^-- related one. Additional studies are required to confirm such hypotheses.

ACKNOWLEDGMENTS

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APPENDICES

APPENDIX I: COMPOSITIONS OF CREAMS 1–3

Table IX
Compositions of Creams 1–3

Cream 1: W/O		Cream 2: W/O		Cream 3: O/W	
Composition	%(w/w)	Composition	%(w/w)	Composition	%(w/w)
F.A	1.00	F.A	1.00	F.A	1.00
Active	0.70	Active	0.80	Active	—
Neutralizing	—	Neutralizing	—	Neutralizing	0.22
Charge	0.20	Charge	—	Charge	—
Preservative	0.60	Preservative	0.70	Preservative	0.95
Fat	—	Fat	—	Fat	12.00
Polymer	11.00	Polymer	4.00	Polymer	8.00
Silicone	24.00	Silicone	26.50	Silicone	13.00
Glycerin	7.00	Glycerin	5.00	Glycerin	7.00
DPPG	—	DPPG	—	DPPG	—
Other solvents	62.50	Other solvents	66.50	Other solvents	64.33
Surfactant	—	Surfactant	0.50	Surfactant	—
Vitamin	—	Vitamin	—	Vitamin	0.50

APPENDIX II: COMPOSITIONS OF SERUMS 4–8

Table X
Compositions of Serums 4–8

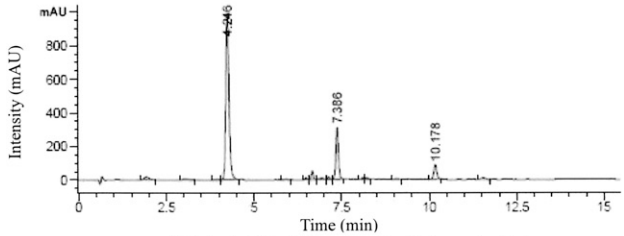
Serum 4: O/W		Serum 5: O/W		Serum 6: O/W		Serum 7: W/Si		Serum 8: Gel System	
Composition	%(w/w)	Composition	%(w/w)	Composition	%(w/w)	Composition	%(w/w)	Composition	%(w/w)
F.A	1.00	F.A	1.00	F.A	1.00	F.A	1.00	F.A	1.00
Active	0.10	Active	—	Active	0.15	Active	0.10	Active	0.15
Charge	—	Charge	—	Charge	—	Charge	0.10	Charge	—
Preservative	0.90	Preservative	0.40	Preservative	0.60	Preservative	0.70	Preservative	0.50
Fat	5.50	Fat	3.00	Fat	0.50	Fat	—	Fat	—
Polymer	1.20	Polymer	1.10	Polymer	4.30	Polymer	2.00	Polymer	0.40
Silicone	—	Silicone	4.75	Silicone	6.00	Silicone	20.10	Silicone	—
Glycerin	7.00	Glycerin	3.00	Glycerin	7.00	Glycerin	23.00	Glycerin	3.00
DPPG	—	DPPG	—	DPPG	7.00	DPPG	—	DPPG	—
Other solvents	89.40	Other solvents	88.70	Other solvents	86.05	Other solvents	76.00	Other solvents	97.10
Surfactant	0.90	Surfactant	0.50	Surfactant	0.90	Surfactant	—	Surfactant	0.85
Vitamin	1.00	Vitamin	0.55	Vitamin	0.50	Vitamin	—	Vitamin	—

APPENDIX III: COMPOSITIONS OF CREAM 9 AND SERUM 10

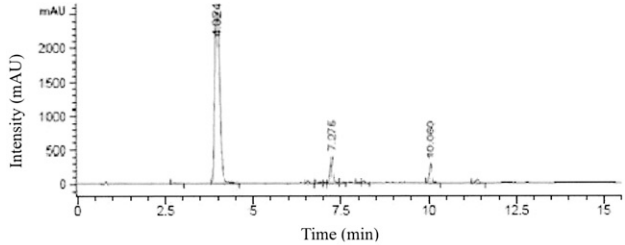
Table XI
Compositions of Cream 9 and Serum 10

Cream 9: O/W		Serum 10: O/W	
Composition	%(w/w)	Composition	%(w/w)
F.A	1.00	F.A	1.00
Active	—	Active	0.10
Neutralizing	0.22	Neutralizing	—
Charge	—	Charge	—
Preservative	0.95	Preservative	0.90
Fat	12.00	Fat	5.50
Polymer	8.00	Polymer	1.20
Silicone	13.00	Silicone	—
Glycerin	—	Glycerin	—
DPPG	30.00	DPPG	30.00
Other solvents	64.33	Other solvents	89.40
Surfactant	—	Surfactant	0.90
Vitamin	0.50	Vitamin	1.00

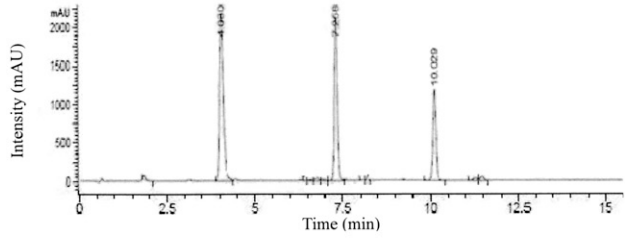
APPENDIX IV: LIQUID CHROMATOGRAM AND MASS SPECTRA RESULTS OF SOLUTIONS A–E (DIFFERENT pH) AND OF SOLUTION D INCUBATED AT RT, 45, 60, AND 75 DEGREES



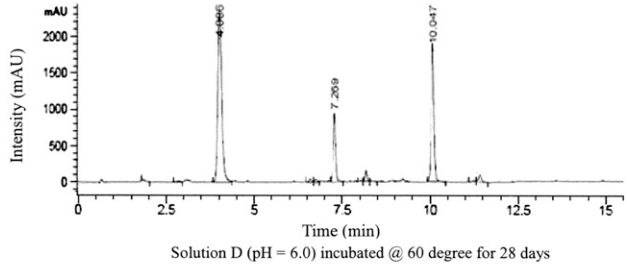
Solution A (pH = 3.0) incubated @ 60 degree for 14 days



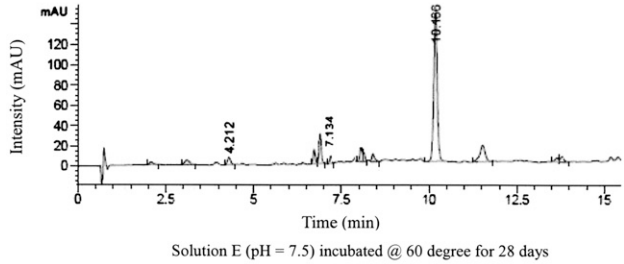
Solution B (Water free) incubated @ 60 degree for 60 days



Solution C (pH = 4.5) incubated @ 60 degree for 28 days



Solution D (pH = 6.0) incubated @ 60 degree for 28 days



Solution E (pH = 7.5) incubated @ 60 degree for 28 days

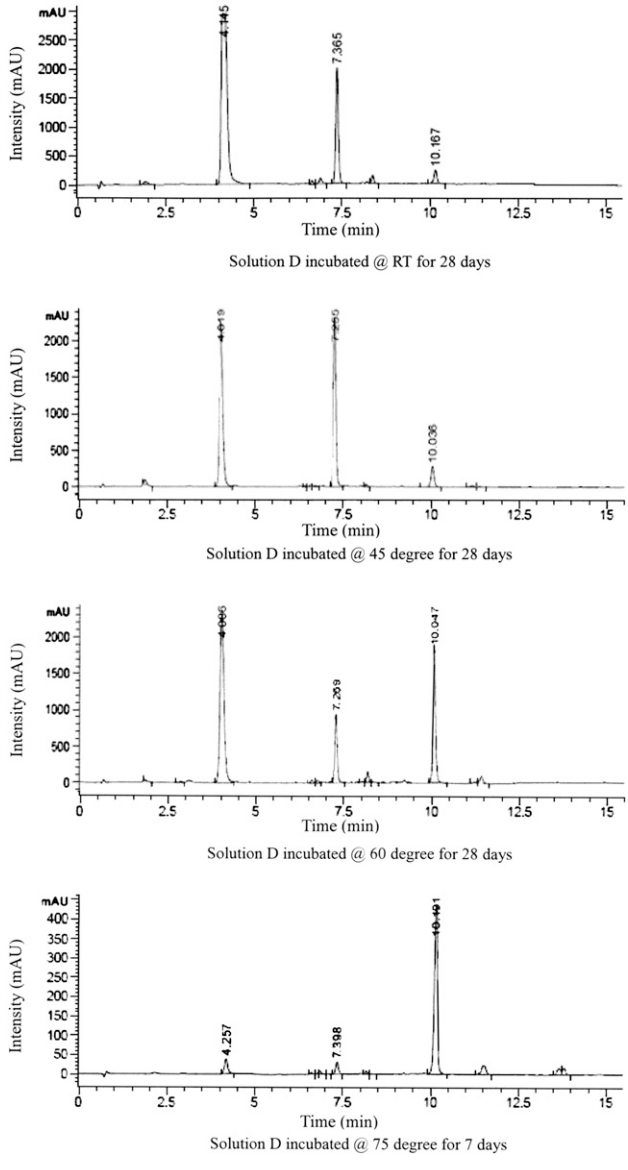


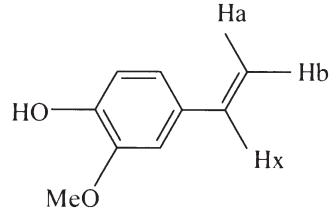
Figure 11. Liquid chromatogram of solutions A–E (different pH) and of solution D incubated at RT, 45, 60, and 75 degrees. The retention times of key peaks in Figure 11 are listed below in Table XII.

Table XII
Retention Time of Key Peaks

Retention time (min)	4.0	7.3	10.0
m/z: [M-]	193	149	299

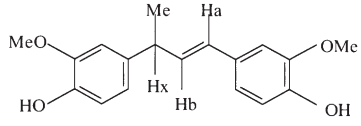
APPENDIX V: SPECTROSCOPIC DATA OF PVG AND ITS DIMER

Compound 1: 4-vinylguaiacol. Colorless to pale yellow oil; nature of TLC was the same as that of a standard 4-vinylguaiacol sample. IR spectra indicate that the molecule has –Ar, –OH, C–O, and C=C groups. Mass spectra (MS) show molecular ion M- as 149, which indicates that the molecular formula is $C_9H_{10}O_2$. This compound was identified as 4-vinylguaiacol; IR cm^{-1}: 3600 (–OH), 3100, 3050, 3000, 2980, 2900 (–Ar), 1620 (C=C), 1280, 1050 (C–O), 930, 790; MS (m/z): 149 (M-), 134; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.92 (3H, s, MeO), 5.12 (1H, dd, Ha), 5.57 (1H, s, OH), 5.62 (1H, dd, Hb), 6.64 (1H, dd, Hx), 6.86-6.94 (3H, m, Ar-H) (6.78 1H, d, Ar-H; 6.86 1H, dd, Ar-H; 7.03 1H, d, Ar-H); UV (methanol): λmax = 220nm, 280nm.



Compound 1: PVG.

Compound 2: 1, 3-bis (4-hyroxy-3-methoxyphenyl) -1-butene. Compound 2 was white acerate crystal, which was crystallized slowly by standing. It could be easily oxidized in air and could not be recrystallized in the usual solvents. The IR spectrum bore a strong resemblance to that of 4-vinylguaiacol except that, notably, the band at 970 cm^{-1} was weaker with respect to the band at 1036 cm^{-1}. IR cm^{-1}: 3600 (–OH), 3100, 3050, 3000, 2980, 2900 (–Ar), 1620 (C=C), 1280, 1050 (C–O), 930, 790; MS(m/z): 299 (M-). Molecular formula: $C_{18}H_{20}O_4$; ^1H NMR (CD_3COCD_3, 300 MHz) δ: 1.19-1.40 (3H, d, Me), 3.52 (1H, m, Hx), 3.83 (6H, s, MeO), 6.28 (1H, dd, Hb), 6.32 (1H, d, Ha), 6.74-7.04 (6H, m, Ar-H); UV (methanol): λmax = 270nm.



Compound 2: Dimer of PVG.