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# Effect of storage temperature on phenolics stability in hawthorn (*Crataegus pinnatifida* var. *major*) fruits and a hawthorn drink

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**Abstract**

The stability of five major phenolics, namely (−)-epicatechin (EC), procyanidin $B_2$ (PC-$B_2$), chlorogenic acid (ChA), hyperoside (HP) and isoquercitrin (IQ), in hawthorn fruits and a canned hawthorn drink were evaluated during 6 months of storage in the dark at three different temperatures (4, 23 and 40 °C). HPLC with a diode-array detector was used to determine the contents of the individual compounds. The results showed that the studied phenolics in the hawthorn fruits and the drink were both stable at 4 °C and relatively unstable at 23 and 40 °C with varied extents of degradation. At room temperature (23 °C), marked degradations of EC and PC-$B_2$ were observed in both the fruits and the drink with around 50% and 30% decrease after a 6-month storage, respectively. A more significant decrease of the phenolics was observed at 40 °C, especially for EC and PC-$B_2$, which were almost completely degraded after a 6-month storage. HP, IQ and ChA were relatively stable at 23 °C, but unstable at 40 °C. Therefore, low-temperature storage is recommended for maintaining the quality and efficacy of hawthorn fruits and its preparations.


*Keywords:* Stability; Hawthorn; (−)-Epicatechin; Procyanidin $B_2$; Chlorogenic acid; Hyperoside; Isoquercitrin

## 1. Introduction

Hawthorn, a common name of all plant species in the genus *Crataegus* of the Rosaceae family, has long been used as herbal medicine, particularly in China and the European countries. It has been used for the treatment of various cardiovascular diseases, including myocardial weakness, paroxysmal tachycardia, hypertension and arteriosclerosis (Chang, Zuo, Harrison, & Chow, 2002). Many recent clinical studies have demonstrated that hawthorn extract show a protective effect on patients with NYHA (New York Heart Association) stages I–II heart failure, hyperlipidemia and other cardiovascular diseases (Tauchert, Ploch, & Hubner, 1994; Weikl et al., 1996). Although phenolics, mainly flavonoids and proanthocyanidins, are recognized as active ingredients of hawthorn (Bahorun, Trotin, Pommery, Vasseur, & Pinkas, 1994; Zhang et al., 2001), they are reported to be pH-sensitive with higher stability obtained under acidic conditions (Zhu, Zhang, Tsang, Huang, & Chen, 1997). Hawthorn fruit contains large amounts of organic acids, such as caffeic, malic, tartaric and citric acids, with a total of 3–6% in the dried fruits (Gao, Feng, & Qin, 1995). Such high level of organic acids is expected to result in an acidic condition in the Hawthorn fruits. However, the stability of these phenolics in hawthorn fruits as well as hawthorn preparations during long-term storage, which is essential for their quality and efficacy, remains unknown. Therefore, the present study aimed to

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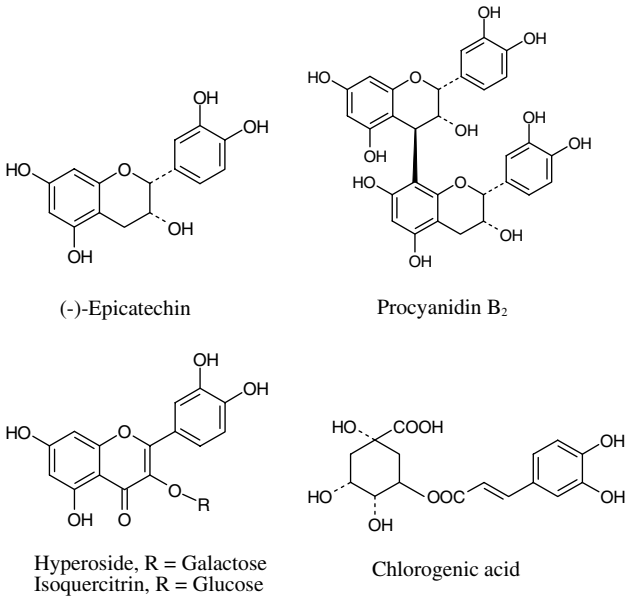


Fig. 1. Chemical structures of the studied hawthorn components.

investigate the stability of the five major phenolics, namely (−)-epicatechin, procyanidin $B_2$, chlorogenic acid, hyperoside (quercetin-3-galactoside) and isoquercitrin (quercetin-3-glucoside) (Fig. 1) in hawthorn fruits and a canned hawthorn drink during a 6-month storage period at three different temperatures (4, 23 and 40 °C).

## 2. Materials and methods

### 2.1. Chemicals and reagents

The standards, (−)-epicatechin (EC) and chlorogenic acid (ChA), were purchased from Sigma (St. Louis, MO, USA), and hyperoside (HP) and isoquercitrin (IQ) were obtained from Carl Roth GmbH (Karlsruhe, Germany). The standard procyanidin $B_2$ (PC-$B_2$) was isolated and purified, by us, from the ethyl acetate fraction of hawthorn fruits 80% ethanol extract, through repeated column chromatography, alternating between a normal phase silica gel column and a reversed-phase ($C_{18}$) silica gel column. The purity of all standards, as tested by HPLC with a photo diode-array detector at the wavelengths ranging from 200 to 400 nm, was found to be better than 99.2%. Acetonitrile (HPLC grade) and ethanol (analytical grade) were obtained from Labscan (Labscan Asia Co. Ltd., Thailand). Distilled and deionized water were used throughout the study.

### 2.2. Hawthorn fruits and a hawthorn drink

Dried slices of hawthorn fruits were purchased from Hong Kong market and the seeds were removed before the experiment. The species of the fruits used was identified as *Crataegus pinnatifida* Bge. Var. *major* N.E. Br. by Prof. Zhong-Zhen Zhao from the School of Chinese Medicine, Hong Kong Baptist University.

A canned hawthorn drink (utilized for clinical studies) was made from the juice produced with the same hawthorn species through the common fruit juice operation by Lee Kum Kee (HK) Food Co. Ltd. in May, 2000, and provided by the Department of Biochemistry at the Chinese University of Hong Kong. The drink contained 8 g fresh hawthorn fruits extract and 15 g sucrose per 100 ml of water. All tested products were from the same batch (No. 20000523) with a pH value of 3.05.

### 2.3. Storage conditions

The hawthorn fruits were kept well-mixed and packed in three polyethylene bags, which were protected from light by wrapping with aluminium foil. These bags, together with the above-mentioned canned hawthorn drinks, were stored for up to 6 months in a refrigerator (4 °C), a cupboard at room temperature (about 23 °C) and an oven (40 °C), respectively. Samples were taken at various time intervals, at 1, 2, 3, 4 and 6 months, and the contents of studied phenolics were analyzed by HPLC.

### 2.4. Sample preparations

Prior to analyses, the freeze-dried fruit sample (about 20 g) was ground to powder by a coffee grinder. The powder (0.5 g) was macerated in 25 ml of 50% ethanol in a tight-capped bottle at room temperature for 4 h, and then sonicated for an additional hour. After centrifugation, 1 ml of the supernatant was evaporated to dryness at 35 °C under reduced pressure. The residue was reconstituted with 0.5 ml of mobile phase (10% acetonitrile in 25 mM $NaH_2PO_4$ buffer, pH 2.4). After centrifugation at 13,000 rpm for 5 min, 50 μl of the supernatant were injected into the HPLC column for analysis. For samples from the canned drink, they were diluted with an equal volume of distilled water, and then centrifuged at 13,000 rpm for 5 min. An aliquot of 50 μl of the supernatant was subsequently analyzed by HPLC. All tested samples were prepared and determined in triplicate.

### 2.5. HPLC analysis

The five phenolics present in the hawthorn fruits and drink were analyzed by HPLC using our previous method (Chang, Zhu, Zuo, Chow, & Ho, 2001). A Beckman Gold HPLC system (Beckman Coulter, Inc., Fullerton, CA, USA), equipped with a 126 solvent delivery module, a 168 photo diode-array detector and a 507e autosampler was used. A $C_{18}$ column

(Radial-pak cartridge, 10 cm × 8 mm I.D., 4 μm particle size, Waters, Milford, MA, USA) was used for separation and eluted by a linear gradient solvent system that consisted of solvents A and B (5% and 25% acetonitrile in 25 mM sodium phosphate buffer, respectively, pH 2.4) with a flow rate of 1 ml/min. The proportion of solvent B was increased from 10% to 80% during the first 20 min, held for 10 min, and then returned to 10% in the last 5 min. The effluent was monitored at 278 nm for PC-$B_2$ and EC, and 360 nm for ChA, HP and IQ. The studied components were quantified using the calibration curves prepared from the standards obtained.

## 3. Results

Fig. 2 shows the HPLC chromatograms of the studied hawthorn fruits extract (in 50% ethanol). The initial contents of the studied phenolics in the hawthorn fruits and the hawthorn drink are shown in Table 1. EC and PC-$B_2$ were the two most abundant components, with 348 and 374 mg/100 g in the fruits, and 22 and 26 mg/100 ml in the drink, respectively.

### 3.1. Stability of the studied phenolics in hawthorn fruits

Stabilities of the five phenolics present in dried hawthorn fruits during the 6-month storage period, at three different temperatures, are shown in Fig. 3. The results show that all studied components were stable at a storage temperature of 4 °C. However, these compounds were unstable at the higher temperature of 40 °C (Fig. 3). Both EC and PC-$B_2$ were found to be extremely unstable and degraded almost completely after a 6-month storage, with only about 3% of initial contents remaining. ChA, HP and IQ were also unstable at 40 °C, with about 40%, 55% and 53% of their initial contents remaining, respectively.

Table 1
Initial contents of the studied compounds in hawthorn fruits and a hawthorn drink

| Compound | Content | |
|---|---|---|
| | Hawthorn fruits (mg/100 g) | Hawthorn drink (mg/100 ml) |
| Procyanidin $B_2$ | 374 ± 1.14 | 25.6 ± 0.41 |
| (−)-Epicatechin | 349 ± 5.10 | 21.9 ± 0.04 |
| Chlorogenic acid | 78.0 ± 0.48 | 5.02 ± 0.02 |
| Hyperoside | 21.8 ± 0.22 | 0.10 ± 0.01 |
| Isoquercitrin | 14.9 ± 0.31 | 0.63 ± 0.01 |

At room temperature (about 23 °C), HP and IQ were relatively stable, with less than 8% loss in the fruits after 6 months of storage. However, EC and PC-$B_2$ were still quite unstable, with about 50% degradation over the same period. ChA exhibited about 30% degradation under the same storage conditions.

### 3.2. Stability of the studied phenolics in a hawthorn drink

The storage stability of the five studied compounds present in the drink is shown in Fig. 4. As in hawthorn fruits, these compounds were all stable at lower temperature (4 °C) and relatively unstable at higher temperatures (23 and/or 40 °C). When the drink was stored at room temperature (23 °C) for 6 months, the percentage of ChA, EC and PC-$B_2$ remaining were about 92%, 71% and 67%, respectively. During storage at 40 °C, the con-

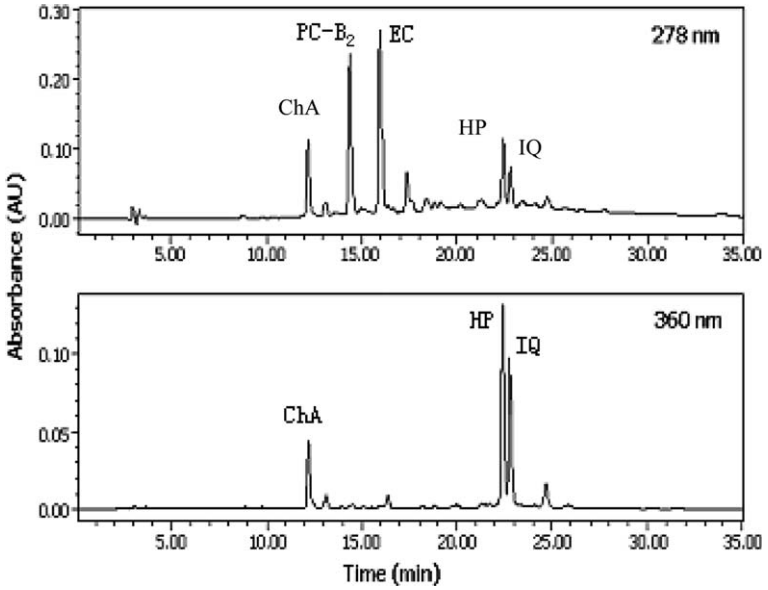


Fig. 2. Representative HPLC chromatograms of hawthorn fruits 50% ethanol extract.

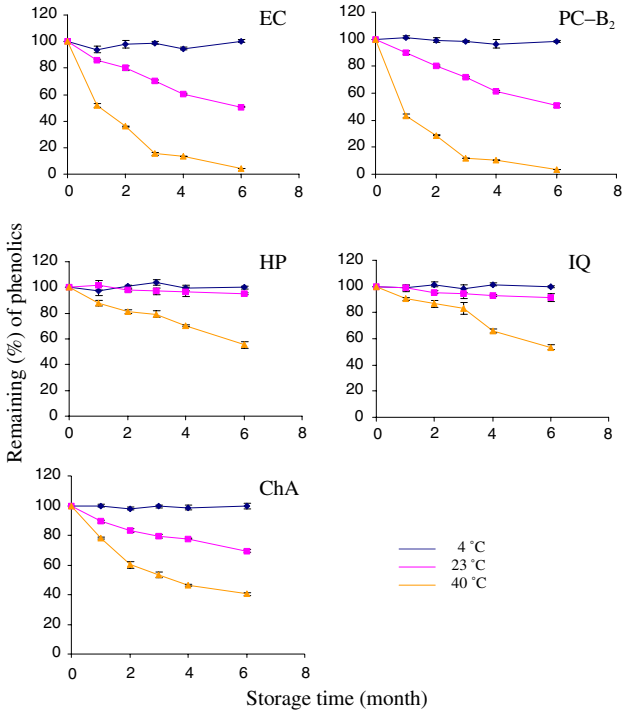


Fig. 3. Stabilities of the major active phenolics, (−)-epicatechin (EC), procyanidin $B_2$ (PC-$B_2$), hyperoside (HP), isoquercitrin (IQ) and chlorogenic acid (ChA), present in hawthorn fruits stored for 1, 2, 3, 4 and 6 months at different temperatures (4, 23 and 40 °C).

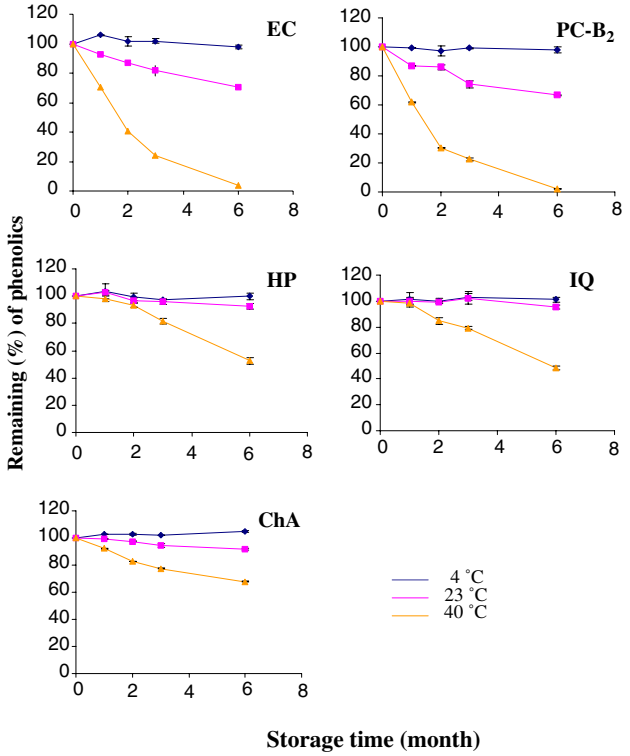


Fig. 4. Stabilities of the major active phenolics, (−)-epicatechin (EC), procyanidin $B_2$ (PC-$B_2$), hyperoside (HP), isoquercitrin (IQ) and chlorogenic acid (ChA), present in a hawthorn drink stored for 1, 2, 3 and 6 months at different temperatures (4, 23 and 40 °C).

tents of ChA, EC and PC-$B_2$ remaining were about 68%, 4% and 2%, respectively. The stabilities of HP and IQ in the hawthorn drink were similar to those in the fruits under the three different temperature conditions.

## 4. Discussion

The effect of pH on the stability of plant phenolics has been extensively investigated in previous studies (Friedman & Juergens, 2000; Zhu et al., 1997). These studies consistently showed that the phenolic compounds, such as chlorogenic acid, caffeic acid, gallic acid, flavonoids, and green tea catechins, were pH-sensitive: the lower the pH, the greater was the stability. For instance, the tea catechins are extremely unstable in alkaline solution (pH > 8) and degrade almost completely in a few minutes, whereas they are very stable in acidic solution (pH < 4) (Zhu et al., 1997).

Hawthorn fruits have long been used in traditional Chinese medicine and European herbal medicine, and are widely consumed as food, in the form of juice, drink, jam and canned fruit. Due to the significant amounts of various organic acids, including caffeic acid, malic acid and tartaric acid, contained in the hawthorn fruits (Gao et al., 1995), the components in hawthorn preparations, such as hawthorn drink, might exist in acidic condition (pH 3.05 for the drink in the current study).

In the present study, temperature was found to markedly influence the stability of the studied compounds, even if they were in acidic conditions in both the fruits and the drink. Therefore, our results further imply that hawthorn herbs and preparations should be stored at relatively low temperatures in order to avoid the degradation of the existing phenolic compounds in the products. We recommend that the storage temperature should be lower than 23 °C, preferably at 4 °C.

Among the five studied compounds, EC and PC-$B_2$ were highly unstable, both at room temperature and 40 °C after 6 months of storage. These results are in agreement with previous findings on green tea catechins in cosmetic formulations (oil/water emulsions). The catechins were found to decrease to 70% of the initial content at room temperature and only a minimum amount remained at 40 °C after 6 months (Frauen, Rode, Steinhart, & Rapp, 2000). Spanos, Wrolstad, and Heatherbell (1990) demonstrated similar findings with a complete degradation of procyanidins, including catechin, epicatechin, and procyanidins $B_1$, $B_2$, $B_3$ and $B_4$, after the storage of concentrated apple juice at 25 °C for 9 months.

Unlike EC and PC-$B_2$, two flavonoids (HP and IQ) were found to be fairly stable. These findings are consistent with the observation from Zafrilla, Ferreres, and Tomas-Barberan (2001), which showed that IQ and

kaempferol 3-glucoside, present in red raspberry jam, decreased only slightly after 6 months of storage.

Moreover, the present study also showed that certain compounds, such as ChA, EC and PC-$B_2$, were much more unstable in the hawthorn fruits than in the hawthorn drink. The reason for the greater degradation of these compounds in the fruits may be due to other chemical changes, such as oxidation by oxygen in the air and decomposition by enzymes present in the fruits. Certain enzymes may be active in the fruits and contribute to the degradation of the active components (Tomás-Barberán & Espín, 2001), whereas these enzymes would be inactivated by the thermal process applied during manufacturing of the hawthorn drink.

Possible degradation pathways of these phenolics may be related to their oxidation, hydrolysis or isomerization, which were suggested from previous studies but not investigated in the present study. EC has been shown to be isomerized into (−)-catechin after being heated at high temperature (>82 °C) for 20 min or stored at 40 °C after a few days (Komatsu et al., 1993; Wang & Helliwell, 2000). In addition, quinones have been suggested as the major degradants of EC and PC-$B_2$ through chemical or enzymatic oxidations (de Gaulejac, Vivas, Nonier, Absalon, & Bourgeois, 2001). HP and IQ, as flavonoid glycosides, may be easily hydrolyzed and converted into their aglycones on heating with water under acidic conditions or by enzymes. ChA is an ester, which may be hydrolyzed to quinic acid in hawthorn fruits or drinks, as has been suggested in the preparation of a coffee beverage (Yamada, Komatsu, & Shirasu, 1997). Moreover, like EC and PC-$B_2$, the phenolic compounds, HP, IQ and ChA may also be oxidized during long-term storage.

## 5. Conclusion

The present study has demonstrated the stability profiles of the five major active phenolics present in the hawthorn fruits and drink. Epicatechin, procyanidin $B_2$, chlorogenic acid, hyperoside and isoquercitrin, are unstable at 23 and 40 °C with greater than 20% degradation in 2–6 months of storage. It is concluded that low-temperature storage is best for hawthorn fruits and its preparations for maintaining the activities of these phenolics.

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## References

Bahorun, T., Trotin, R., Pommery, J., Vasseur, J., & Pinkas, M. (1994). Antioxidant activities of *Crataegus monogyna* extracts. *Planta Medica, 60*, 323–328.

Chang, Q., Zuo, Z., Harrison, F., & Chow, M. S. S. (2002). Hawthorn. *Journal of Clinical Pharmacology, 42*, 605–612.

Chang, Q., Zhu, M., Zuo, Z., Chow, M. S. S., & Ho, W. K. K. (2001). A high performance liquid chromatographic method for simultaneous determination of Hawthorn active components in rat plasma. *Journal of Chromatography B, Biomedical Sciences and Applications, 760*, 227–235.

de Gaulejac, N., Vivas, N., Nonier, M. F., Absalon, C., & Bourgeois, G. (2001). Study and quantification of monomeric flavan-3-ol and dimeric procyanidin quinonic forms by HPLC/ESI-MS. Application to red wine oxidation. *Journal of the Science of Food and Agriculture, 81*, 1172–1179.

Frauen, M., Rode, T., Steinhart, H., & Rapp, C. (2000). Determination of green tea catechins by HPLC/EIS-MS – A method for stability analysis of green tea extracts in cosmetic formulations. *Lebensmittelchemie, 54*, 141–142.

Friedman, M., & Juergens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry, 48*, 2101–2110.

Gao, G. Y., Feng, Y. X., & Qin, X. Q. (1995). Analysis of the chemical constituents of hawthorn fruits and their quality evaluation. *Yaoxue Xuebao, 30*, 138–143.

Komatsu, Y., Suematsu, S., Hisanobu, Y., Saigo, H., Matsuda, R., & Hara, K. (1993). Studies on preservation of constituents in canned drinks. Part II. Effects of pH and temperature on reaction kinetics of catechins in green tea infusion. *Bioscience, Biotechnology, and Biochemistry, 57*, 907–910.

Spanos, G. A., Wrolstad, R. E., & Heatherbell, D. A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry, 38*, 1572–1579.

Tauchert, M., Ploch, M., & Hubner, W. D. (1994). Effectiveness of hawthorn extract LI 132 compared with the ACE inhibitor captopril. Multicentre double-blind study with 132 NYHA stage II heart failure patients. *Munch Medizinische Wochenschrift, 136*, 27–33.

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture, 81*, 853–876.

Wang, H., & Helliwell, K. (2000). Epimerisation of catechins in green infusions. *Food Chemistry, 70*, 337–344.

Weikl, A., Assmus, K. D., Neukum-Schmidt, A., Schmitz, J., Zapfe, G., Noh, H. S., et al. (1996). *Crataegus* Special Extract WS 1442. Assessment of objective effectiveness in patients with heart failure. *Fortschritte der Medizin (NYHA II), 114*, 291–296.

Yamada, M., Komatsu, S., & Shirasu, Y. (1997). Changes in components of canned coffee beverage stored at high temperature. *Colloque Scientifique International sur le Cafe, 17*, 205–210.

Zafrilla, P., Ferreres, F., & Tomas-Barberan, F. A. (2001). Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *Journal of Agricultural and Food Chemistry, 49*, 3651–3655.

Zhang, Z., Chang, Q., Zhu, M., Huang, Y., Ho, W. K. K., & Chen, Z. Y. (2001). Characterization of antioxidants present in hawthorn fruits. *Journal of Nutritional Biochemistry, 12*, 144–152.

Zhu, Q. Y., Zhang, A., Tsang, D., Huang, Y., & Chen, Z. Y. (1997). Stability of green tea catechins. *Journal of Agricultural and Food Chemistry, 45*, 4624–4628.