Catechin composition of *C. sinensis* plants cultivated in Galicia (NW Spain)

Salinero M.C.[1]*, Vela P.[1], Barreiro R.[1-2], Pérez-Santín E.[2-3]

[1] Estación Fitopatolóxica Areeiro, Deputación de Pontevedra, Subida á Carballeira, 36153 Pontevedra, Spain; [2] Departamento de Química Orgánica, Universidade de Vigo, CINBIO, Lagoas-Marcosende, 36310 Vigo, Spain; [3] Universidad Internacional de la Rioja, Av. de la Paz, 137, 26004 Logroño, Spain;

Email: carmen.salinero@depo.es

Abstract

The beneficial health effects of tea have been described and studied for centuries. Today, it is widely known that these health benefits are related to catechins, secondary metabolites of plants that are included in a group of polyphenols (flavonoids) present in high quantities in different kinds of tea. They are also responsible for the antioxidant capacity of tea. The presence of these compounds and their derivatives in leaves of different clones of *C.sinensis* was confirmed in previous studies carried out at the Estación Fitopatolóxica Areeiro (Pontevedra). Five selected clones cultivated in different conditions (sun and shadow) were studied, and the aqueous extracts of their fresh young leaves were obtained in order to determine their catechin composition.

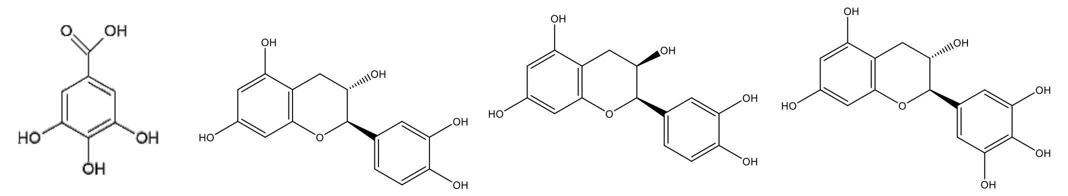
Keywords: tea plant, different culture conditions, HPLC-UV

INTRODUCTION

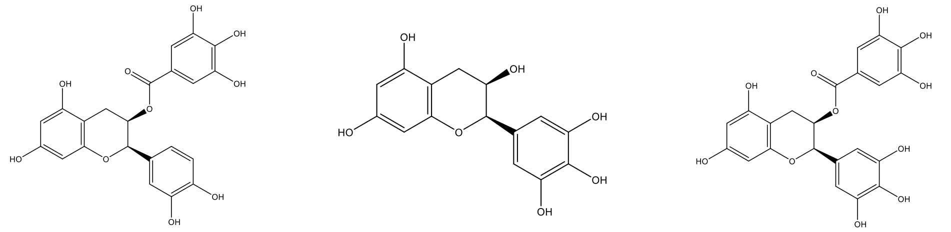
Since its early history, tea consumption has been associated with numerous health benefits. This fact has encouraged, especially during the last decades, the proliferation of studies carried out by scientific researchers from various fields aimed at knowing their components and applications.

Thanks to this research, today it is widely known that this drink is an important and very rich source of polyphenols, particularly a subgroup of compounds included in the family of flavonoids known as catechins. The total catechin content of tea varies depending on the leaf age, the tea type and the mode of preparation; but, in general, they all contain catechin (C), epicatechin (EC), gallocatechin (GC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG), being the last one the most abundant.

Catechins belong to the flavonoid family, one of the five in which polyphenols are classified. They are a series of secondary plant metabolites with a colorless appearance, which are astringent and water soluble. Its molecular structure is composed of two or more benzene rings with different numbers of hydroxyl groups (Figure 1).



Gallic acid (GA) (+)-Catechin (C) (-)-Epicatechin (EC) (+)-Gallocatechin



(-)-Epicatechin-3-gallate (-) Epigallocatechin (-)-Epigallocatechin-3-gallate

Figure 1. Structure of the main catechins present in tea.

The oxidation of these compounds occurs easily, so they are excellent antioxidants widely used in the food industry to preserve all types of food and retard the appearance of the rancidity of fats and oils. However, not all catechins have the same antioxidant capacity, since this property varies inversely to the oxidation potential of the molecule, which depends on the chemical structure of each of them.

The importance of tea catechins relies not only on the property that makes them suitable for improving food preservation, but also that they have numerous benefits for human health. They help control weight, prevent cardiovascular diseases, have anticancer activity, improve oral health and reduce iron levels in people with hemochromatosis, among others.

The mechanism by which catechins contribute to weight control was explained by European researchers in a paper published in 1999. In this study, through trials with volunteers, they found that prolonged consumption of green tea extracts with 90 mg of EGCG, energy expenditure increased, and the respiratory quotient of 10 healthy young men decreased 24 hours after intake. Later, in research carried out in 2007 in Japan that including more than 100 people, it was shown that daily consumption of catechins for 12 weeks favored weight loss.

Scientific works developed in the last decades, in different countries, corroborate the capacity of catechins to prevent the occurrence of coronary diseases and the reduction of the incidence of heart attacks and mortality caused by these types of pathologies. In fact, tea consumption (with a high content of this group of flavonoids) combined with a diet low in saturated fats is related with low rates of heart disease registered among the population of Japan.

Different *in vitro* and epidemiological studies confirmed the anti-cancer potential of tea polyphenols and describe their mode of action. Some studies suggest that regular consumption of green tea can reduce the incidence of a variety of cancers, including colon, pancreatic, and stomach cancers, and other diseases.

Another benefit of tea catechins is related to oral health. A study in England carried out with more than 6,000 children proved that drinking tea is associated with low levels of tooth decay. The mechanism through which this occurs was explained in other scientific papers that found that oolong tea polyphenols inhibit the adhesion of bacteria to the teeth surface by reducing streptococcus hydrophobia. Moreover, they curb the appearance of tooth decay by decreasing acid production.

Besides the mentioned beneficial effects, tea flavonoids decrease iron absorption in patients with genetic hemochromatosis, a disease that affects iron metabolism and causes its accumulation. This ability was demonstrated in a study carried out in Germany with 18 individuals with this pathology. For several weeks, individuals accompanied the meals with tea instead of water, and experienced a considerable reduction of the frequency in the practice of phlebotomies.

In spite of being highly beneficial compounds for our health, humans do not naturally produce catechins (or any other polyphenol), so their incorporation into the body is only made by eating food that contains them. Therefore, in the era of healthy living habits, daily consumption of tea with a high content in catechins is a must.

Plant growth and development is regulated by environmental parameters. In this way, light influences the biosynthesis of many compounds such as flavonoids. Some studies have determined different catechins composition in tea obtained from *C.sinensis* plants grown under sun and shade. The role of catechins is generally considered as providing protection of plants from the damage from UV rays in sunlight, and catechin production is seriously affected by photosynthesis.

The objective of this work was to develop an analytical method to evaluate the catechin composition of fresh young leaves of plants belonging to an experimental plantation of clones of *C.sinensis* in the province of Pontevedra. Comparison between the catechin composition of plants cultivated in different conditions was also carried out.

MATERIALS AND METHODS

Plant material: Young leaves from five selected *Camellia sinensis* clones cultivated in different conditions (sun and shadow) were collected in June 2019 (Figure 1). All plants were grown at the Estación Fitopatolóxica Areeiro (Pontevedra, Spain), with sprinkler irrigation and controlled fertilizer conditions. The five-year-old specimens at the sun are in pots, while those grown in semi-shade are planted directly in the ground under a 60% shade mesh and are almost ten years old. The selected clones and their origins are described in Table 1.

Chemicals and reagents: All reagents used were HPLC grade. Methanol and Acetonitrile were purchased from Fisher Scientific (USA) and Milli-Q water was obtained using a Milli-Q system (Millipore). Standards of catechins were obtained from Sigma-Aldrich (USA) and Extrasynthese (France).

Figure 1. *Camellia sinensis* plants cultivated in the shadow (left) and in the sun (right).

Table 1. Selected clones of *Camellia sinensis* and their origins.

Clone	Origin
EFA-4-Fu	Vietnam
EFA-13-Mo	Moreira Nursery (Fene, A Coruña, Spain)
EFA-104-Mo	Moreira Nursery (Fene, A Coruña, Spain)
EFA-106-Mo	Moreira Nursery (Fene, A Coruña, Spain)
EFA-122-Mo	Moreira Nursery (Fene, A Coruña, Spain)

Extraction: For catechin extraction, 1 g of fresh leaves were weighed and 100 mL of Milli-Q water were added. Samples were infused at 100 ºC for five minutes with continuous stirring. Extracts were cooled and filtered (0.45 µm pore size filter of PTFE) prior to injection in the HPLC-UV.

HPLC: A Varian 920-LC, equipped with a 250 x 4.6 mm x 5 µm C18 SunFire column (Waters) and a Diode Array Detector (DAD) was used. Following the method described by COI/T.20/Doc. nº 24, 2001; the separation and identification of the compounds were carried out with a ternary mobile phase which comprised water 1% V/V formic acid (A) and acetonitrile 1% V/V formic acid (B). The workflow was 1 mL/min for 82 minutes and the injection volume 20 µL. The compounds were detected at 234 nm and 280 nm and they were properly identified using the retention time and concordance of their spectrum at the appropriate wavelength with commercial standards. All samples were analyzed in triplicate obtaining low standard deviation values in all cases.

Calibration line: To determine the concentration of the studied compounds, calibration lines were performed with a standard mixture containing: gallic acid (GA), catechin (C), epicatechin (EC), gallocatechin (GC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). For the working standard solutions, the standard was diluted with methanol to obtain five standard solutions with concentrations in the range of 10-100 mg/Kg. The calibration lines used to quantify each catechin content of the samples studied were obtained by adding the areas of the chromatographic peaks of all standards. All of them had a regression coefficient (R^2) up to 0.99.

RESULTS

All the samples studied presented a high catechin content. As an example, the HPLC-UV chromatogram (280 nm) of clone EFA-4 is illustrated in figure 2, identifying a total of eight peaks corresponding to seven different types of catechins and the caffeine.

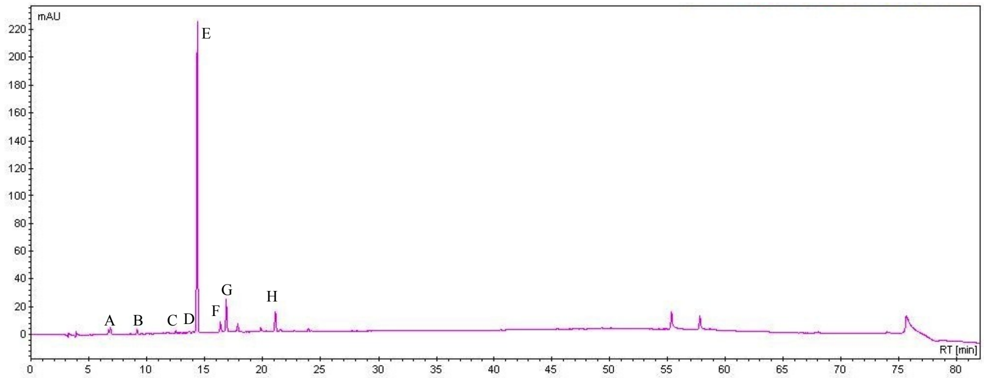


Figure 2. HPLC-UV (280 nm) chromatograms of clone EFA-4. Letters indicate each identified compound. A gallic acid, B gallocatechin, C epigallocatechin, D catechin, E caffeine, F epicatechin, G epigallocatechin-3-gallate and H epicatechin-3-gallate.

All samples of plants cultivated in the sun contained a lower quantity of total catechins (TC) than the samples growing in the shadow. The TC obtained varied from 32 to 46 mg/g and from 24 to 39 mg/g for leaves of plants cultivated in the shadow and in the sun, respectively. Significant differences between the clones studied in different conditions were also observed (Figure 3). Thus, in clones AFA-122 and EFA-104, differences in TC of more than 18 mg/g of fresh leaves were observed between sun and shade grown plants, while in EFA-4 cultivars, the difference in TC was less than 0.1 mg/g of fresh leaves.

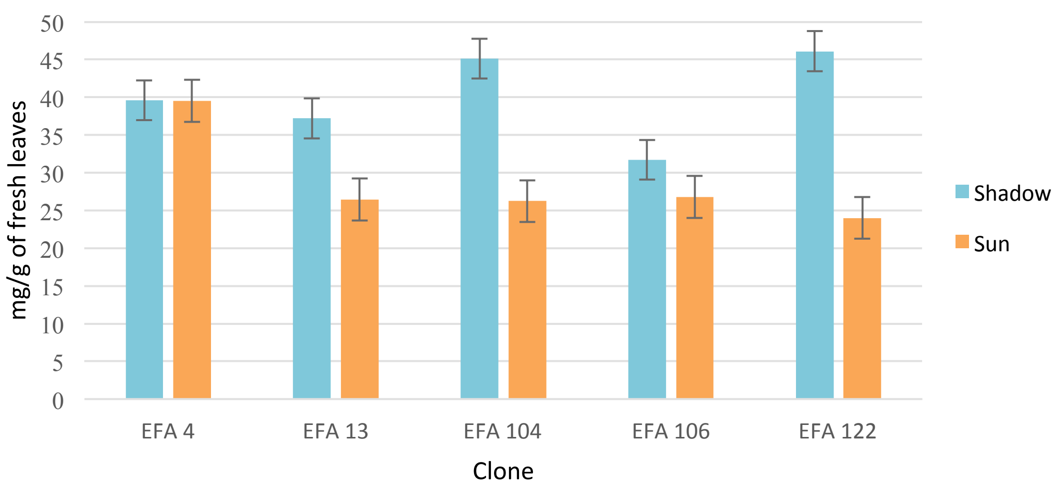


Figure 3: Total catechin content of all samples from *C.sinensis* in different cultivated conditions.

The composition of the catechin content of each sample was obtained. Figures 4 and 5 contain charts with the results of the five clones studied. A clear difference is observed between the two types of samples, as the plants cultivated in the shadow show significantly higher catechin quantities than the plants cultivated in the sun.

Moreover, a variability between the distribution of the content of each catechin was also detected, maybe because the sunlight affects differently the synthesis of each one. For the plants in the shadow, there is no clone that clearly has the highest or lowest values and only short variations between the position of EGCG and epigallocatechin were observed when comparing the clones. In the case of the plants in the sun, the clone EFA-4 has the highest values of all catechins, gallic acid has the lower catechin content for all clones and little differences were observed among the catechin content of the other compounds.

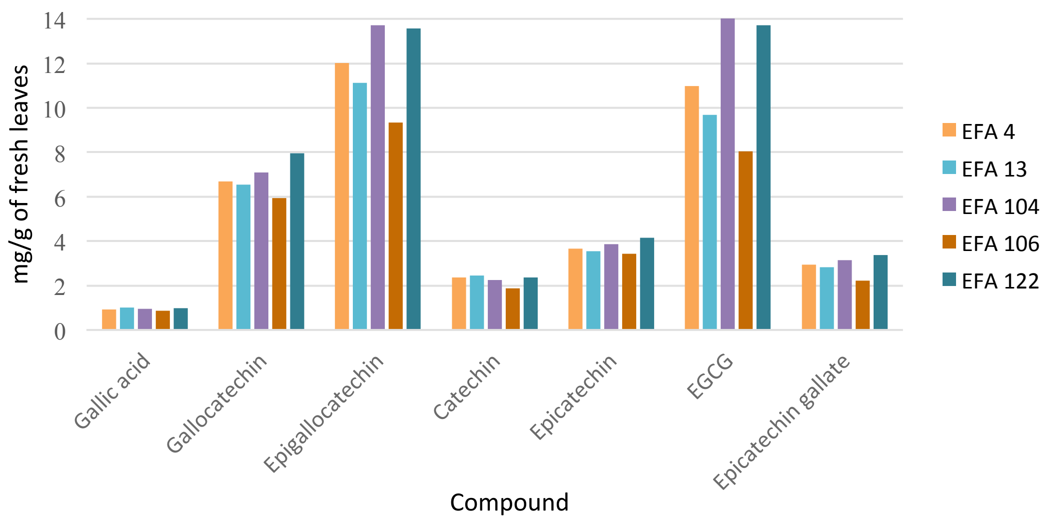


Figure 4: Catechin composition of all samples from *C. sinensis* cultivated in the shadow.

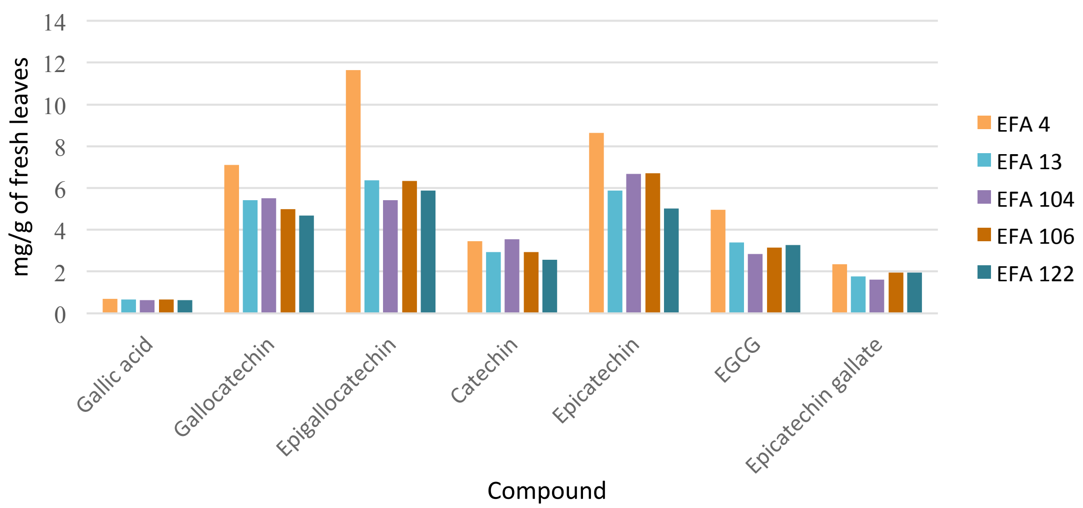


Figure 5: Catechin composition of all samples from *C. sinensis* cultivated in the sun.

CONCLUSIONS

An efficient extraction and analysis method by means of HPLC-UV to determine and quantify seven catechins (gallic acid, catechin, epicatechin, gallocatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate) in plant extracts from *C.sinensis* leaves cultivated in Galicia (Spain) was established in the present work.

The method developed has proved suitable for the determination of the catechin composition and total catechin content present in the plant material of *C.sinensis* cultivated at the Estación Fitopatolóxica Areeiro. Moreover, it was observed that the catechin profile of the different clones and cultivated conditions showed significant differences, being greater the catechin content in the EFA-4 clone and in all plants cultivated in the shadow.

All clones studied presented very high total catechin values.

Although some authors suggest that the cultivation of tea plants in the sun increases the amount of caffeine in the leaves and decreases the levels of catechins, the results obtained showed that in our area, the ideal plant cultivation condition to produce catechins is in the shade, as there are significant differences in catechin levels with respect to plants cultivated in the sun.

After studying the different clones, interesting differences have been observed among them. This feature opens the door to continue their analysis, and to select the best clones or plants to be used as a possible natural source of catechins.

REFERENCES

Ahmad N., Mukhtar H. 1999. Green tea polyphenols and cancer: biologic mechanisms and practical implications. Nutrition reviews, 57(3):78-83.

Alexis A.F, Jones V.A., Stiller M.J. 1999. Potential therapeutic applications of tea in dermatology. International Journal of Dermatology, 38:735–743.

Ananingsih V.K., Sharma A., Zhou W. 2013. Green tea catechins during food processing and storage: a review on stability and detection. Food Research International, 50(2):469-479.

Balentine D.A., Wiseman S.A., Bouwens L.C.M. 1997. The chemistry of tea flavonoids. Critical Reviews in Food Science and Nutrition, 37:693–704.

COI/T.20/Doc. nº 24. 2001. Consejo Oleícola Internacional, Preparación de los ésteres metílicos de los ácidos grasos del aceite de oliva y el aceite de orujo de oliva.

Dreostic I.E., Wargovich M.J., Yang C.S. 1997. Inhibition of carcinogenesis by tea: the evidence from experimental studies. Critical Reviews in Food Science and Nutrition, 37: 761–770.

Dulloo A. G., Duret C., Rohrer D., Girardier L., Mensi N., Fathi M., Vandermander, J. 1999. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. The American journal of clinical nutrition, 70(6):1040-1045.

Graham H. N. 1992. Green tea composition, consumption, and polyphenol chemistry. Preventive medicine, 21(3): 334-350.

Grzesik M., Naparło K., Bartosz G., Sadowska-Bartosz I. 2018. Antioxidant properties of catechins: Comparison with other antioxidants. Food chemistry, 241:480-492.

Hertog M. G., Kromhout D., Aravanis C., Blackburn H., Buzina R., Fidanza F., Pekkarinen M. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of internal medicine, 155(4):381-386.

Jones C., Woods K., Whittle G., Worthington H., Taylor G. 1999. Sugar, drinks, deprivation and dental caries in 14-year-old children in the north west of England in 1995. *Community dental health*, 16(2):68-71.

Kaltwasser J.P., Werner E., Schalk K., Hansen C., Gottschalk R., Seidl C. 1998. Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis. *Gut*, 43(5):699-704.

Matsumoto M., Minami T., Sasaki H., Sobue S., Hamada S., Ooshima T. 1999. Inhibitory effects of oolong tea extract on caries–inducing properties of Mutans streptococci. Caries research, 33(6):441-445.

McKay D. L., Blumberg J. B. 2002. The role of tea in human health: an update. *Journal of the American College of Nutrition*, 21(1):1-13.

Mariya J.K.M., Sasikumar R., Balasubramanian M., Saravanan M., RajKumar R. 2003. Influence of light on catechin biosynthesis in tea. Tea 24:80–86.

Nagao T., Hase T., Tokimitsu I. 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity, 15(6):1473-1483.

Nakachi K., Matsuyama S., Miyake S., Suganuma M., Imai, K. 2000. Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 13(1-4):49-54.

Nadiah N.I., Uthumporn U. 2015. Determination of Phenolic and Antioxidant Properties in Tea and Spent Tea Under Various Extraction Method and Determination of Catechins, Caffeine and Gallic Acid by HPLC, International Journal on Advanced Science, Engineering and Information Technology, Vol. 5, (3):158-164.

Setiawan V.W., Zhang Z.F., Yu G.P., Lu Q.Y., Li Y.L., Lu M.L., Wang M.R., Guo C.H., Yu S.Z., Kurtz R.C., Hsieh C.C. 2001. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. International Journal of Cancer, 92:600–604.

Song, R., Kelman, D., Johns, K. L., & Wright, A. D. (2012). Correlation between leaf age, shade levels, and characteristic beneficial natural constituents of tea (*Camellia sinensis*) grown in Hawaii. *Food Chemistry*, 133(3), 707-714.

Vinson J. A., Dabbagh Y. A., Serry M. M., Jang J. 1995. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry 43(11):2800-2802.

Wang H., Provan G.J., Helliwell K. 2003. HPLC determination of catechins in tea leaves and tea extracts using relative response factors. Food Chemistry 81:307-312.

Wang Y.S., Gao L.P, Shan Y., Liu Y.J., Tian Y.W., Xia T. 2012. Influence of shade on flavonoid biosynthesis in tea (*Camellia sinensis* (L.)O. Kuntze). Scientia Horticulturae 141:7–16.

Wehrwein P. 1999. More evidence that tea is good for the heart. The Lancet 353(9150), 384.

Yang C. S., Chung J. Y., Yang G. Y., Chhabra S. K., Lee M. J. 2000. Tea and tea polyphenols in cancer prevention. The Journal of nutrition, 130(2):472S-478S.