

UDC 54.03 + 547.978.4

Stability of Green Tea Catechins in the Solid Phase and Aqueous Solutions

I. O. LOMOVSKY^{1,2}

¹Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences, Ul. Kutateladze 18, Novosibirsk 630128 (Russia)

E-mail: Lomovsky@solid.nsc.ru

²Novosibirsk State University, Research and Education Centre for Molecular Design and Ecologically Safe Technologies, Ul. Pirogova 2, Novosibirsk 630090 (Russia)

(Received June 1, 2011; revised June 22, 2011)

Abstract

The stability of green tea catechins was studied in the solid phase and in aqueous solution in the course of storage, degradation rate values were determined depending on ambient conditions. The stability of catechins was investigated in the course of mechanical activation; possibility is demonstrated for obtaining a mechanocomposite consisting of plant raw material and ascorbic acid, with a higher stability.

Key words: green tea catechins, stability, mechanochemical activation, mechanocomposite

INTRODUCTION

Adverse external factors, such as unhealthy environmental conditions, radioactive and UV radiation exposure, smoking and alcohol abuse result in an increased level of free radical concentration in a human organism. The connection between the increased levels of free radical concentration in an organism and the development of many diseases is an established fact. For the prevention of diseases it is proposed to introduce antioxidants into the diet (food, beverages, and dietary supplements). Using the antioxidant substances of natural origin those represent mainly polyphenolic compounds is considered to be most safe [1].

Among beverages, tea, of course, is leading concerning the use. Tea leaves contain 6 to 16 mass % of compounds those belong to a polyphenolic series (catechins), among those prevail epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallo-

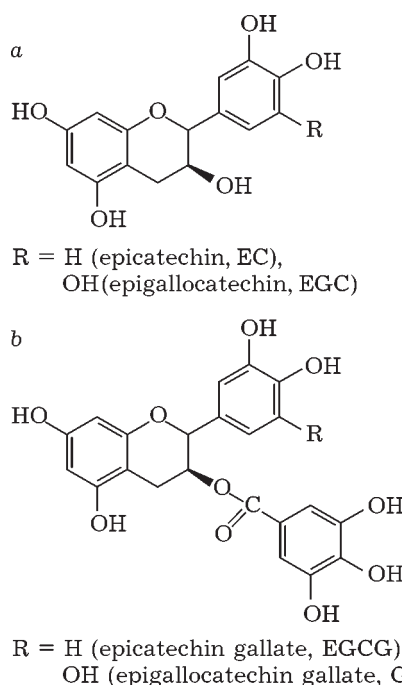


Fig. 1. Structural formulae: a – epicatechin and epigallocatechin, b – epicatechin gallate and epigallocatechin gallate.

catechin gallate (EGCG). The structural formulae are presented in Fig. 1. To all appearance, catechins can be considered to be one of the most large-capacity naturally occurring antioxidant resources.

Catechins are highly reactive compounds; the most among them actively enter into the reactions of epimerization and oxidation [2]. In this regard, when developing the products with a high content of catechins, particular attention should be focused on oxidation processes and the processes of degradation those could occur within the raw materials in the course of storage and treatment.

Obtaining the antioxidants from plants is mainly based on multiple extraction by solvents with different polarity. According to the chemical nature, antioxidant substances represent reducing agents to interact in a relatively ready manner with oxygen and other oxidizers. The technical result of the oxidation reactions in the course of extraction with water or other liquid solvents consists in decreasing the yield of these substances and in difficulties in obtaining species those are stable with respect to storage.

In recent years, mechanochemical methods are widely used in the processing of food raw materials, the products of plant cultivation and animal husbandry, commercially cultivated microorganisms, wild-growing plants. The reactions of main constituents of plant raw materials as it follows are under investigation: polysaccharides (cellulose, hemicellulose, and pectin), polyphenolic components (lignin and humic substances), vegetable proteins and lipids. Mechanochemical methods are used in the processing of plant raw and other biogenic raw materials in order to produce biologically active substances such as alkaloids, organic acids [3] and glycosides [4], *i. e.*, the components whose content in the vegetable raw material is much less than the content of main components (usually at a level of several percent).

Many labile bioactive substances including gallocatechins inherent in green tea are more stable in the solid phase than in the liquid one. However, the relative stability of gallocatechins in the liquid and solid phases were almost not studied, although solid-phase methods appeared rather promising in the preparative chemistry of a number of organic compounds [5, 6].

It is demonstrated that the isolation and storage of many volatile antioxidants is much more efficient in the presence of the second, more stable antioxidant such as ascorbic acid [7].

The purpose of the work consisted in studying the mechanical activation of catechin extraction process from vegetable raw materials as well as the effect of adding the antioxidant (ascorbic acid) on this process, both in an aqueous solution or in the solid phase.

EXPERIMENTAL

Reagents and materials

As a raw material we used green tea (engineering specifications TU 9191-00300570186-04, DagomysChay Ltd., Russia), analytical grade ascorbic acid, catechol >99 % (Alfa Aesar Co.).

Mechanical activation

A weighed sample portion of green tea was treated in a centrifugal activator mill AGO-2 (Novits Co., Novosibirsk) under the acceleration of milling bodies amounting to 200, 400, and 600 m/s². The treatment time ranged within 1–2 min.

Obtaining the aqueous extracts

A weighed sample portion of the plant raw material was extracted during 1 h with water at a mass ratio between the solid and liquid phases equal to 1 : 1000 at a room temperature. The extract was filtered using a 0.45 μm filter in order to use for further analyzing.

Storage in air

The extract obtained was held under permanent stirring in a beaker, under air and light access. At certain time, we took samples for analysis.

Storage without air

The extract was stored in a sealed flask without air access under light. At certain times, samples were taken for analysis.

Storage in the solid phase

The sample was stored in a vacuum bag with the access of light. At certain times samples were taken to perform water extraction.

HPLC analysis

The analysis was performed using a Milichrom A-02 high-performance liquid chromatograph (Econova Co., Novosibirsk). As a sorbent we used ProntoSil 120 5-C18. The elution was performed in a gradient mode, double distilled water being used as eluent A, acetonitrile being used as eluent B. The detection was performed photometrically at a wavelength equal to 290 nm.

Data processing

Each experiment was conducted in five replicates. For the kinetic data obtained for each point we calculated the degradation reaction constant in the pseudo first order approximation, according to equation

$$k = \ln([C_0]/[C])/t$$

where C is the current concentration at time t ; C_0 is the initial concentration calculated by

means of the method of least squares. The confidence interval was calculated for a confidence probability level equal to 0.9.

RESULTS AND DISCUSSION

Kinetics of catechin degradation in solution

The degradation of the catechins in aqueous solution was investigated earlier (see *e. g.*, [7]). The content of main and minor components in the extract can vary depending on the starting materials. Our samples contained 150 μg of total catechins in 1 mL of the aqueous extract including EGC 60 mg/mL, EGCG 70 mg/mL, ECG 10 mg/mL. Kinetics was studied for the degradation of catechins, depending on air access to the solution (Fig. 2, *a*). In the absence of air the degradation exhibited slowing down (see Fig. 2, *b*), however with no completely stopping, which indicates the presence of other oxidant compounds in the extract.

In aqueous solution under air access, at 20 °C and neutral pH the total concentration of catechins exhibits a 7 to 9-fold decrease within 6 h due to the degradation of the main active ingredients such as EGCG and EGC. The introduction of stabilizers such as ascorbic acid, re-

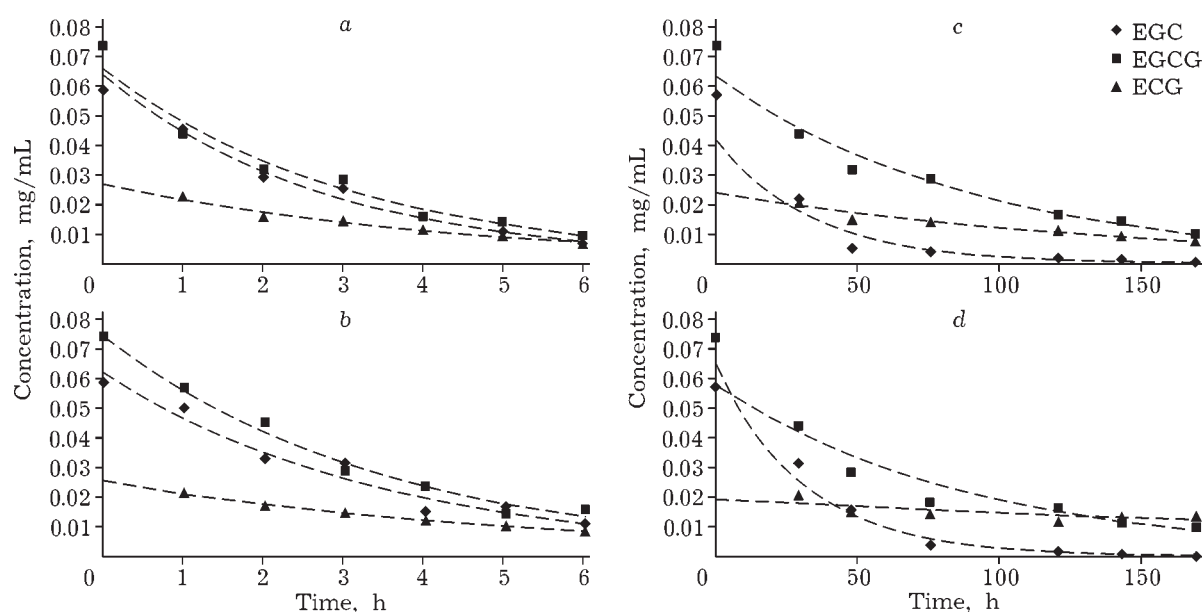


Fig. 2. Kinetic curves for the degradation of catechins in aqueous extract: *a* – under access for air, *b* – the same in the presence of ascorbic acid (1 mg/mL), *c* – in the absence of air, *d* – the same in the presence of ascorbic acid (1 mg/mL).

sults in a slight decrease in the catechin degradation rate (see Fig. 2, b). The results obtained are in a good agreement with the literature.

Thus, in this case the minor components have a little effect on the degradation process in air, which process could include a wide range of reactions. It is believed that the main process consists in the generation of corresponding radicals in the reactions between the catechins and oxygen [8] with the subsequent polymerization of catechins. In this regard, we carried out experiments concerning the storage of water extracts in the absence of air.

The degradation of catechins in an aqueous solution free of oxygen and air takes place, too (see Fig. 2, c). First of all the reaction is entered by the most reactive components (EGCG and EGC). The duration of 90 % conversion exhibits an increase from 6 to 96 h for EGC from 6 to 260 h for EGCG, the remaining components demonstrated the degradation level less than 35 % within 96 h. Adding the ascorbic acid causes the rate of the reaction to decrease (see Fig. 2, d). The conversion level equal to 90 % can be reached after 530 h for EGCG and after 340 h for EGC, the other components after 340 h exhibit the degradation level lower than 40 %.

Thus, it is demonstrated that the degradation of catechins in the green tea extracts occurs with no access for air, too. In this case, the degradation could occur due to the epimerization, oxidation and polymerization with the other components of the extract, such as phenolic acids [9, 10]. Changing the oxidation mechanism is also indicated by changing the ratio between the rate constants. With no air access the fastest degradation is observed for EGC, whereas under air access the most reactive component is presented by EGCG. In this case, no significant changes in the degradation rate of the components are observed with adding ascorbic acid. To all appearance, the process does not occur according to a free radical mechanism. The authors of [11] demonstrated that many bottled teas exhibit the catechin content to be ten times less than that in fresh extracts; this fact could be most likely explained by the degradation processes with no access for air.

Degradation kinetics of catechins in the solid phase

The stability of catechins in the solid phase within the extract depends on the ambient humidity level and additives in the mixture [12]. The degradation is accelerated by atmospheric humidity level greater than 40 % at 22 °C. In the case of such ambient humidity level the water content in the initial solid extract exceeds 10 %. To all appearance, this value could correspond to the formation of the aqueous phase, wherein the processes of rapid degradation occur. However, owing to a small amount of water bulk they occur much more slowly than the degradation in solution. Moreover, in this case the ascorbic acid plays a role of a preoxidant and accelerates the degradation of catechins.

The process of catechin degradation in the initial plant raw material was investigated. Owing to the absence of aqueous phase in the raw material, the chosen value of the ambient humidity level was equal to 20 %. The humidity level of the initial raw material amounted to 6.7 %. It is demonstrated that in the solid phase the degradation of catechins is a very slow process (Fig. 3, a). The degradation rate of the mechanical mixture of plant raw material and

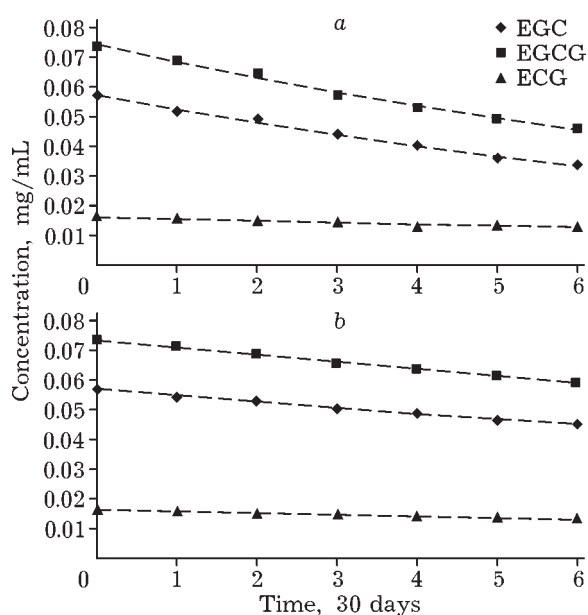


Fig. 3. Kinetic curves for the degradation of catechins in the solid phase within the plant raw material (a) and within mechanocomposite with 10 mass % of ascorbic acid (b).

TABLE 1

Rate constants for the degradation of catechins in the solid and liquid phases in the approximation of pseudo-first kinetic order, s^{-1}

Experimental conditions	ECG	EGCG	ECG
In aqueous extract with air access	$(8.7 \pm 0.7) \cdot 10^{-5}$	$(10 \pm 1.5) \cdot 10^{-5}$	$(8.0 \pm 0.9) \cdot 10^{-5}$
In the solution of ascorbic acid (0.1 mg/mL), under air access	$(7 \pm 1) \cdot 10^{-5}$	$(7.7 \pm 0.6) \cdot 10^{-5}$	$(5.2 \pm 0.2) \cdot 10^{-5}$
In aqueous extract with not access for air	$(9.7 \pm 1.5) \cdot 10^{-6}$	$(3.9 \pm 0.5) \cdot 10^{-6}$	$(2.1 \pm 0.2) \cdot 10^{-6}$
In the solution of ascorbic acid (0.1 mg/mL) with no access for air	$(8 \pm 1) \cdot 10^{-6}$	$(4.3 \pm 0.6) \cdot 10^{-6}$	$(1.7 \pm 0.6) \cdot 10^{-6}$
In the solid phase within the plant raw material	$(3.5 \pm 0.3) \cdot 10^{-8}$	$(3.0 \pm 0.2) \cdot 10^{-8}$	$(1.6 \pm 0.1) \cdot 10^{-8}$
In the solid phase, a mechanical mixture of plant raw material and ascorbic acid (10 mass %)	$(3.2 \pm 0.3) \cdot 10^{-8}$	$(3.1 \pm 0.2) \cdot 10^{-8}$	$(1.6 \pm 0.1) \cdot 10^{-8}$
In the solid phase, mechanical activation with ascorbic acid (10 mass %)	$(1.5 \pm 0.1) \cdot 10^{-8}$	$(1.4 \pm 0.1) \cdot 10^{-8}$	$(1.3 \pm 0.2) \cdot 10^{-8}$

ascorbic acid does not differ from the degradation rate in the initial raw material. This could be explained by the fact that the degradation processes are local in nature. Thus, since the solid phase diffusion is extremely difficult, the addition of solid ascorbic acid does not affect the process.

The degradation was studied for catechins in a mechanically activated mixture of green tea and ascorbic acid (see Fig. 3, b). In the course of the mechanical activation the rate of degradation increases, but adding the ascorbic acid causes slowing the degradation in the course of storage. In the course of mechanical activation, a so called mechanocomposite is formed [13], wherein the number of contacts between the particles of green tea and ascorbic acid is great; the particles of ascorbic acid are coating the particles of green tea in the mechanocomposite thereby preventing the catechins from oxidation.

Comparative analysis of the kinetic data

The degradation of the catechins represents a multi-stage process and includes several parallel reactions and diffusion processes at the least, however the kinetic curves are well described by pseudo-first-order kinetic equation. Corresponding rate constants are presented in Table 1.

It can be seen that the degradation rate of catechins in the solid phase in the samples of powder preparations under investigation are 1000 times lower than it is observed for the solution extracts of these samples.

In solution, the ascorbic acid causes the process of degradation to slow down, but only to a slight extent.

In the solid phase the mechanical mixture of ascorbic acid and green tea does not in stability from the original green tea in stability. In order to slow the degradation in the solid phase it is required that the ascorbic acid to form a mechanocomposite with green tea. In this case, the degradation rate of EGCG and EGC exhibits halving.

CONCLUSION

1. The use of solid-state mechanochemical processes allows one to reduce the loss of catechins in the course of the manufacturing operations producing the antioxidant preparations of green tea.

2. The antioxidants of green tea are about 1000 times more stable in the solid phase (in the raw materials, in mechanocomposites obtained *via* joint treatment with ascorbic acid) as to compare with antioxidants in solutions.

3. Obtaining a stable mechanocomposite is provided by mechanical treating the mixture of green tea and ascorbic acid at the acceleration of acting bodies equal to 20 g during 2 min.

Acknowledgements

The author expresses his sincere gratitude to his scientific mentors Prof. O. I. Lomovsky and Associate Prof. A. A. Politov.

REFERENCES

- 1 Minina S. A., Kaukhova I. E., Khimiya i Tekhnologiya Fitopreparatov, GEOTAR-Media, Moscow, 2009.
- 2 Vuong Q. V., Golding J. B., Nguyen M., Roach P. D., *J. Sep. Sci.*, 33 (2010) 3415.
- 3 Korolev K. G., Lomovsky O. I., Rozhanskaya O. A., Vasiliev V. G., *Chem. Nat. Comp.*, 39, 4 (2003) 366.
- 4 Lomovsky O. I., Boldyrev V. V., Mekhanokhimiya v Reshenii Ekologicheskikh Problem, Izd-vo GPNTB, Novosibirsk, 2006.
- 5 Boldyrev V. V., *J. Mater. Sci.*, 39 (2004) 5117.
- 6 Tanaka K., Toda F., *Chem. Rev.*, 100, 3 (2000) 1025.
- 7 Chen Z., Zhu Q., Wong Y., Zhang Z. and Chung Y., *J. Agric. Food Chem.*, 46 (1998) 2512.
- 8 Nanjo F., Goto K., Seto R., Suzuki M., Sakai M., Hara Y., *Free Radical Biol. Med.*, 21 (1996) 895.
- 9 Wang R., Zhou W., Jiang X. 2008., *J. Agric. Food Chem.*, 56 (2008) 2694.
- 10 Tanaka T., Mine C., Watarumi S., Fujioka T., Mihashi K., Zhang Y.-J., Kouno I., *J. Nat. Prod.*, 65 (2002) 1582.
- 11 Chen Z., Zhu Q., Tsang D., Huang Y., *J. Agric. Food Chem.*, 49 (2001) 477.
- 12 Ortiz J., Ferruzzi M. G., Taylor L. S., Mauer L. J., *J. Agric. Food Chem.*, 56 (2008) 4068.
- 13 Lomovsky O. I. (Ed.), in: Mekhanokompozity – Prekursory dlya Sozdaniya Materialov s Novymi Svoystvami, Izd-vo SO RAN, Novosibirsk, 2010.