IT'S TOO EASY TO JUST SAY **"BREWED TEA"!**

hot water and tea leaves
are the only ingredients allowed

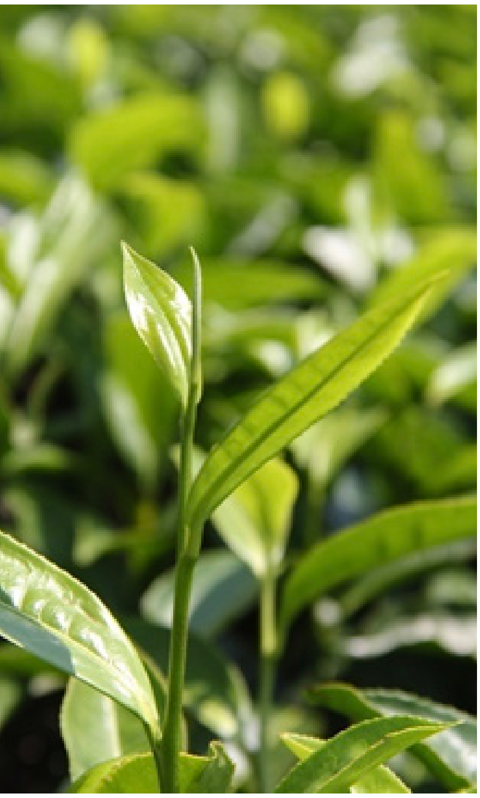
l'italiana aromi

AROMI DAL 1890
FLAVOURS SINCE

Since 1988, when the first ice-tea drink bottled in PET in Europe was formulated in the R&D Laboratory at L'Italiana Aromi, such drinks have been rapidly gaining market shares, first in Italy then all over the world.

Around 2006, when we felt traditional ice tea market had reached its maturity, we started developing a new tea drink concept: ready-to-bottle brewed tea. Brewed tea has been gaining popularity among consumers, because it is prepared as a traditional home made tea infusion and therefore has the same organoleptic profile.

It is prepared brewing Ceylon tea leaves in hot water for 5 minutes. The high polyphenols content and the colour come exclusively from the tea leaves. After the production we add sugar, ascorbic acid and the flavours. No other industrial processes are applied to produce our brewed tea.



Brewed tea: the ice-tea drink evolution

Our recipes allow the finished product standardization, independently from the chosen packaging system (PET, tetrapak, glass, cans) and from the chosen bottling facility.

Advantages:

- No need of an in-line infusion plant
- Same bottling process as for traditional ice tea drinks
- No experience required in the infusion cycle nor in the raw material purchase
- No waste material management (exhausted tea leaves)
- Our brewed tea can be delivered to any bottling company

AT THIS TIME OUR PRODUCTION CAPACITY CAN REACH THE 150 TONS PER DAY

BREWED TEA

A study made at L'Italiana Aromi

Introduction

This study aims at classifying the main soluble constituents of a traditional home-made **black tea** infusion taking into consideration the following categories: poliphenols, phenolic acids and methyl-xantines.
For this study black tea leaves (Camellia sinensis) from different world origins have been used.

Tea composition

The main soluble constituents of the tea leaves are:

1 polyphenols

- catechins
- flavonols
- flavons
- theaflavins and thearubigins

2 phenolic acids

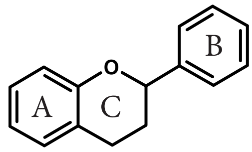
- gallic acid

3 methyl-xanthines

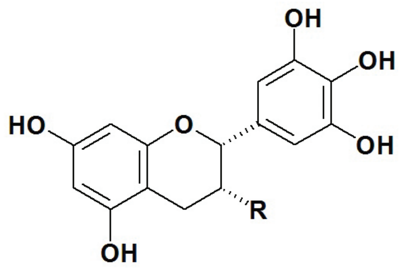
- caffeine
- theobromine
- theophylline

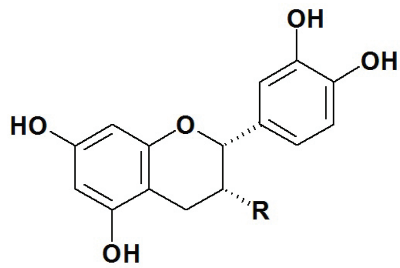
POLYPHENOLS

The tea polyphenols have a C6-C3-C6 skeleton, where the two aromatic rings (A and B), each bearing at least one hydroxyl, are connected by a 3-carbon chain. The three carbons, combined with 1 oxygen and 2 carbons of the A ring, form a third 6-atom ring (C).



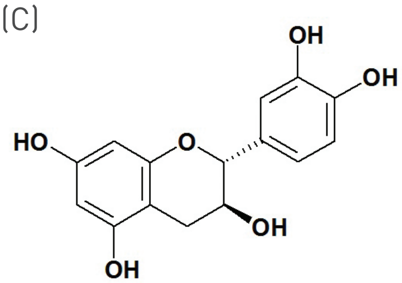
→ **CATECHINS**: among all polyphenols the catechins are the main components and, thanks to their beneficial effects, make tea a concentrate of precious substances. From the organoleptic point of view, catechins give a great contribution to the bitter and astringent taste of tea. These molecules with the polyphenols skeleton **C6-C3-C6** are bound with OH groups. The most abundant catechins, naturally contained in the tea, are the ones listed below. Among them EGCg, the most active and carrying the highest beneficial effects, is also the one that is present in the highest quantity in non fermented tea (green tea).



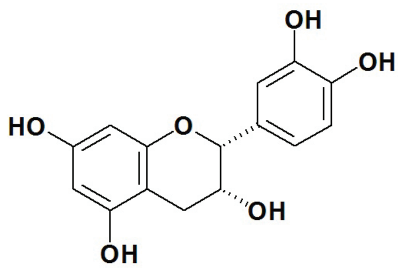


(-)-ECg

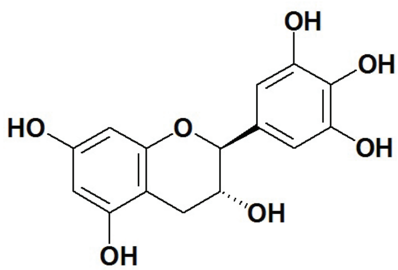
(-)-epigallocatechin-3-gallate (EGCg)
(-)-epigallocatechin (EGC)
(-)-epicatechin-3-gallate (ECg)
(-)-epicatechin (EC);
(-)-gallocatechin (GC)
(+)-catechin (C)
R = gallate



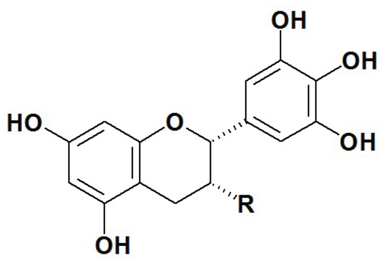
(+)-C



(-)-EC

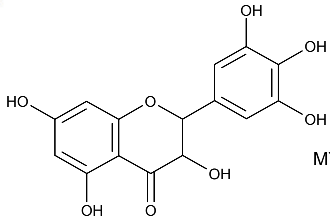


(-)-GC

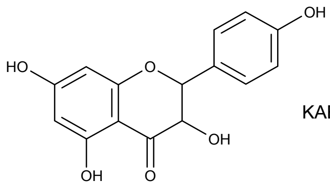


(-)-EGCg

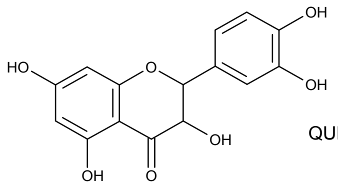
→ **FLAVONOLS**: The flavonols have a **C6-C3-C6** skeleton with **C=O** groups and are present as aglycones only in traces. They are found mainly in the glycosidated form. The most important aglycones are the quercetin, the kaempferol, and the myricetin.
The sugars bound to the flavonols are glucose, rhamnose, galactose and fructose.
Below the structure of these molecules is shown.
Because of their yellow colour, the flavonols together with the theaflavine and the thearubigine contribute to the colour of black tea infusion, while flavonols alone give colour to green tea infusions.



MYRICETIN

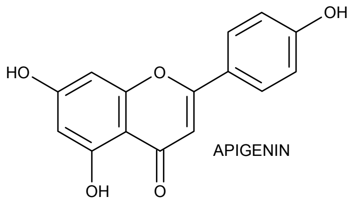


KAEMPFEROL



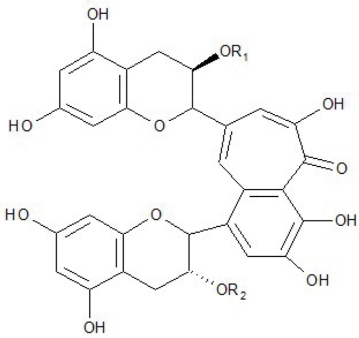
QUERCETIN

→ **FLAVONS**: they are present in low concentration and usually in a glicosidated form (C6-C3-C6 skeleton + C=O groups, position 3 free).
The structure of the most important aglycon of this class, is apigenin, that has not been taken into consideration in this study because of its negligible content.



APIGENIN

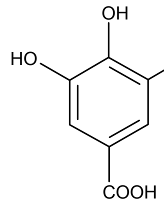
→ **THEAFLAVINS AND THEARUBIGINS**: during the fermentation process of the black tea, the catechins are transformed into THEAFLAVINS_(TF) and THEARUBIGINS_(TR, HPS) by the polyphenoloxidase (PPO) enzim through an oxidative polimerization.
The theaflavins include a certain number of fractions called theaflavin, theaflavin monogallate and theaflavin digallate.
The theaflavins are orange-red substances which contribute significantly to the astringency, vivacity, brilliancy and to the colour of the tea infusion.
Thearubigins and the highly polimerized substances (HPS) derive from the condensation of the oxidated catechins with theaflavins, are another result of the fermentation. The HPS and the TR contribute to the colour, to the palate and to the body of the tea infusion. This substances have not been isolated in a pure form yet, therefore their structure is still to be explained.



R1=R2=H	theaflavin
R1=H, R2=galloil	theaflavin-3-gallate
R1=galloil, R2=H	theaflavin-3'-gallate
R1=R2=galloil	theaflavin-3,3'-gallate

PHENOLICS ACIDS

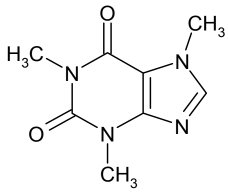
The most representative among the phenolic acids both in green and black tea is the gallic acid. Its structure is given below. The quantity of gallic acid increases during the fermentation as it separates from the catechines gallate.



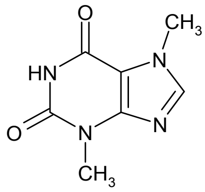
GALLIC ACID

METHYL-XANTHINES

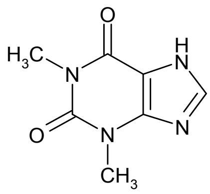
The methyl-xanthines are the caffeine, the theobromine and the theophylline whose structure is depicted below. Theophylline is not easily quantifiable because it is present in traces.



CAFFEINE



THEOBROMINE



THEOPHYLLINE

Experimental phase

We analyzed the most important constituents of a **black tea** infusion as catechins among polyphenols, fenolic acids and methyl-xantines. The infusion has been prepared as a classic home-made preparation of tea leaves in hot water for few minutes. We chose tea leaves grade "fannings" as the ones of the tea bags commonly sold on the market. These leaf types come from different countries that all together represent over 80% of the entire black tea world production. The origins considered are Sri Lanka, India, China, Indonesia, Vietnam and Africa.

Sample preparation
In a glass flask weigh 1.5g tea leaves and add 200g water at 90°C. Stir and let the leaves in static infusion for 5'. Filter on a sieve and when temperature reaches 40°C take the brewed liquid, add the ISTD and analyse immediately.

Analytical standards
Gallic Acid, EGCg, EC, GC, C, Cg, ECg, Hydroxyethyl Theophylline, Caffeine, Theobromine, Vanillic acid (Sigma-Aldrich). EGC, ECg, (Extrasynthese).

Solvents and Reagents
Acetic acid, Water, ACN and Ethanol for HPLC (Sigma-Aldrich).

Preparation of the standard solutions
The standard solutions have been accurately weighed on an analytical scale.
The stock solutions of the various analytes have been prepared and then diluted to the required concentration with the internal standard solutions (ISTD).

Table 1 shows in detail the solvents used in the dilutions and the ISTD of each analyte.

ANALYTE	DILUTION SOLVENT	INTERNAL STANDARD
GALLIC ACID	stock solution: EtOH follow: asSorbic aid 0.1% in water	Vanillic Acid
CATECHINS	stock solution: EtOH follow: ascorbic acid 0.1% in water	Vanillic Acid
METHYL-XANTHINES	stock solution: EtOH/H_2O 1/1 follow: H_2O	7-β-OH-ethyltheophylline

Analytical Method
Different modern analytical methods can be used to classify traditional tea infusions. These methods include GC-MS, LC-MS, NMR, HPLC with UV detector. For this study we standardized an LC-MS method for the chemical characterization of gallic acid, catechins, and methyl-xantines. The analysis have been performed with an Agilent HPLC, series 1260 connected to an Agilent mass spectrometer, model 6120.
The samples have been separated on a KINETEX C18 PHENOMENEX 50 x 2.1mm, 2.6µm.
The quantitative and qualitative determination of these substances has been made with the introduction of an ISTD.

HPLC Conditions

Mobile Phase: A = Water + Acetic acid 0.1%
B = Acetonitrile + Acetic acid 0.1%

Gradient:

TIME (MIN.)	MOBILE PHASE A (%)	MOBILE PHASE B (%)
0	96	4
1	96	4
7	76	24
14	68.5	31.5
15	40	60
20	40	60
22	96	4

Post time: 15 min (equilibration time before new injection)
Flow: 0.2 mL/min
column Temperature: 30°C
Injection volume: 3 μL

MS Parameters

Ionization Mode: API-ESI
Drying Gas Temperature: 350°C
Drying gas Flow: 10 L/min
Nebulizer Gas Pressure 50 psi
VCap+: 4000 V
VCap-: 3000 V
Acquisition Mode: SIM
Polarity: Negative for catechins
Positive for xantines

COMPOUND	M/Z (-)	FRAGMENTOR
Gallic acid	169	80
Vanillic acid (ISTD)	167	80
GC-EGC	305	90
C-EC	289	80
ECg-Cg	441	100
EGCg-GCg	457	100
Theobromine	181	100
Theophylline	181	100
Caffeine	195	100
Hydroxyethyltheophylline (ISTD)	225	100

Table 2 SIM Parameter (the molecules written in blue are our ISTD)

The calibration curves have been built on 3 levels and for each single level 3 repeats have been made. All infusions have been prepared three times by the same technician and each infusion has been injected three times. All values indicated in the tables are the average of the results of all the repeats.

Results

Tables 3 summarize the determinations made for each tea type (results of 3 repeats); the quantity in ppm of total catechins, methyl-xantines and gallic acid. The ratio between total catechin and gallic acid and between caffeine and theobromine can be of interest as well.

Black Tea Leaves	Total Catechins	Caffeine	Theob-romine	Theo-filline	Gallic Acid	Ratio Catechins/ Gallic Acid	Ratio Caffeine/ Theob-romine
ASSAM 1	22	206	13	traces	22	1,0	15,3
MALAWI	24	141	7	traces	17	1,4	19,1
VIETNAM	25	137	5	traces	13	1,8	30,4
ASSAM 2	33	214	16	traces	18	1,8	13,3
ASSAM 3	34	227	16	traces	20	1,7	13,8
ASSAM 4	54	252	26	traces	15	3,7	9,9
CHINA STD	55	135	4	traces	19	2,9	33,9
KENYA	57	153	11	traces	16	3,5	33,9
CEYLON 1	63	150	10	traces	16	4,0	15,0
ASSAM 5	64	225	19	traces	16	4,0	11,7
CEYLON 2	66	115	7	traces	15	4,6	16,4
SUMATRA	68	174	11	traces	13	5,1	16,2
CEYLON 3	70	213	20	traces	26	2,7	10,6
CEYLON 4	81	122	14	traces	21	3,8	9,0
JAVA	83	131	7	traces	19	4,5	18,0
CEYLON 5	108	120	10	traces	13	8,0	12,5
CEYLON 6	126	144	8	traces	16	8,0	17,3
CEYLON 7	156	263	25	traces	36	4,3	10,5

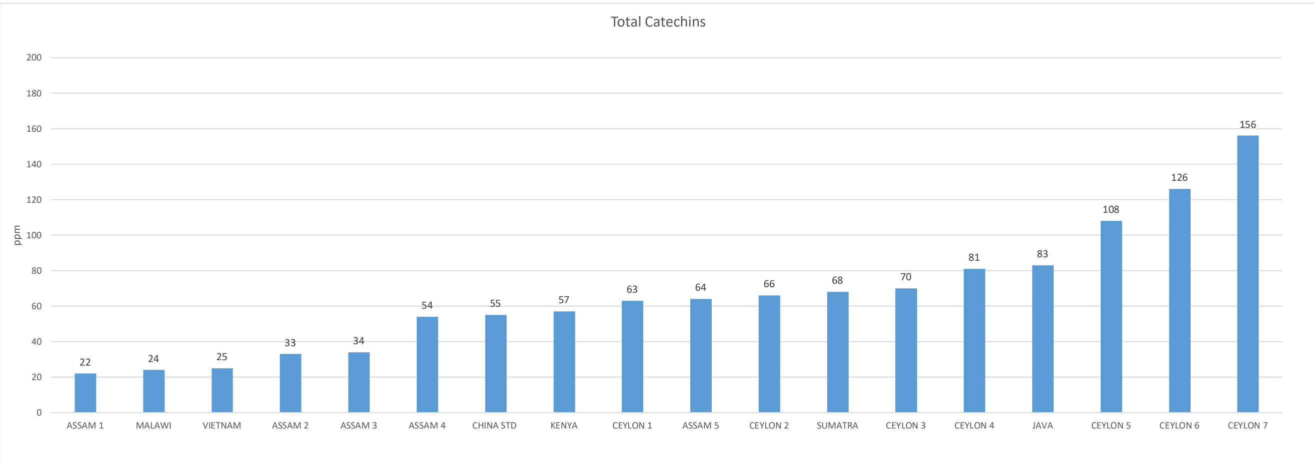
Table 3: total catechins, caffeine, theobromine, gallic acid (as ppm), ration total catechins/ gallic acid and caffeine/theobromine

CATECHINS	22 ÷ 156
CAFFEIN	115 ÷ 263
THEOBROMINE	4 ÷ 26
GALLIC ACID	13 ÷ 36
THEOFYLLINE	traces
CATECHINS/GALLIC ACID	always ≥ 1 (between 1 ÷ 8)
CAFFEINE/THEOBROMINE	between 9 ÷ 34

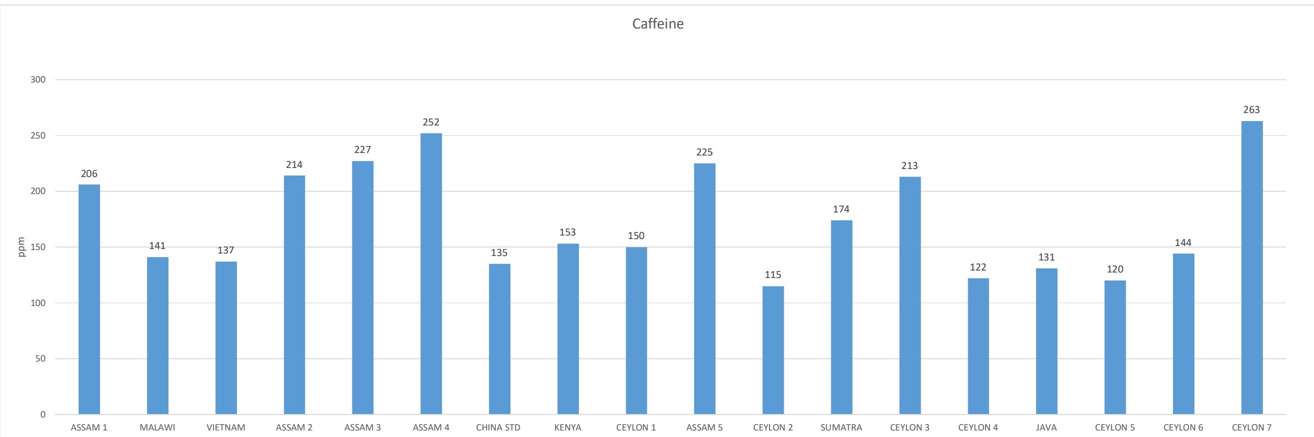
Table 4: amount of main soluble ingredients in black tea leaves analysed and some ratios

CONCLUSIONS

From our study it clearly emerges that all black tea infusions prepared in the traditional way (home-made preparation with a tea bag) and examined by us show a significant **catechins** concentration, between 22 and 156 ppm. Ceylon teas have commonly higher catechins content compared to all the other origins. Among the tea soluble constituents, catechins have the highest beneficial effects and can be considered the real parameter of the good quality of tea leaves and represent the most important parameter to identify the presence of tea.



Caffeine concentration is less variable in all the samples compared to catechins and not always indicate the quality of the black tea leaves. Poor catechins black tea leaves can show at the opposite majors caffeine content among the sample analysed.



The concentration of **gallic acid** is between 13 and 36 ppm. The ratio between black tea total catechins and gallic acid is always higher than 1 even in the case of the black tea types with a lower catechins content.

Theobromine is always present with concentration levels between 4 and 26 ppm while **theophylline** is present only in traces. The ratio between caffeine and theobromine has never been found exceeding 34.

Flavored black tea based beverages **(Ice Tea)** are gaining larger market shares. These soft drinks can be produced using different types of tea extracts (commonly powder or soft tea extracts), products which therefore are subjected to industrial operations of concentration, evaporation, addition of chemical additives to improve the solubility in cold water (sodium hydroxyde, carbonates etc.) and carriers such as maltodextrines. The colour of the finished beverage is obtained usually with powder tea extracts or with the addition of colorants such as caramel, or other natural extracts with a secondary coloring power (e.g. malt, chicory, acorn etc.).

More recently alongside Ice Tea, other flavored beverages based on black tea infusion and generally defined as **"Brewed Tea"** or **"Tea from real infusion"** or **"Real Tea"** are being produced. In their industrial formulation such products reflect the traditional home-brewed preparation. Regardless of geographical origin or leaf grade of the raw material this new tea beverage must consequently contain a significant amount of catechins in the same proportions on average of a traditional home-brewed preparation. It must maintain as well the characteristic aftertaste of tea leaves brewed in hot water. In these beverages the color must derive solely and exclusively from the direct infusion of the black tea leaves and the tea infusion can not undergoes to any other industrial chemical or physical process as it happens for powder and paste tea extracts (evaporation, chemical additives, etc.). The manufacturing process remain the simple infusion of the tea leaves in hot water.

GLUTEN FREE

VEGETARIAN

ALLERGENE FREE

KOSHER

HALAL (PENDING)

VEGAN

l'italiana aromi va

L'Italiana Aromi srl
Carate Brianza (MB) ITALY
tel. +39.0362.990053
www.litalianaaromi.it

MORE INFORMATIONS sales@litalianaaromi.it