Control of functional ingredients in tea (*Camellia sinensis*)

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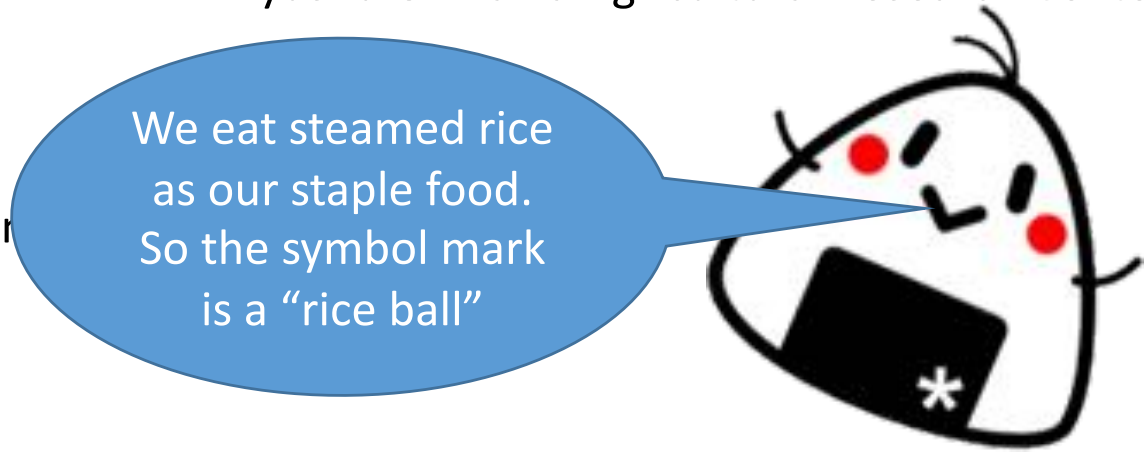
About NARO

NARO means **N**ational **A**griculture Food **R**esearch **O**rganization.

NARO is the core institute in Japan for conducting research and development on agriculture and food.

NARO consists of these centers and institutes.

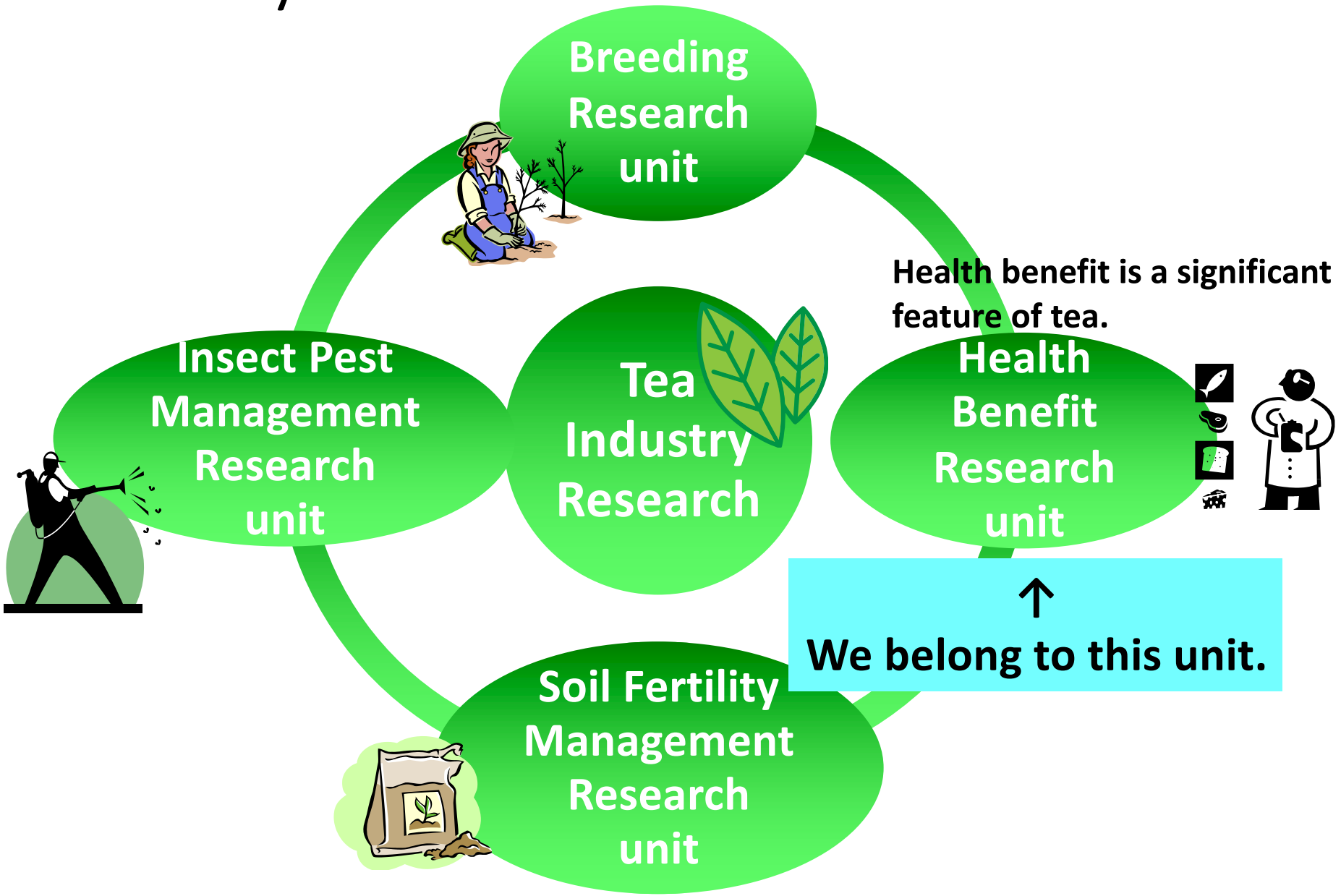
Institute of Fruit Tree and Tea Science,
Institute of Vegetable and Floriculture Science,
Institute of Livestock and Grassland Science,
National Institute of Animal Health,
Institute for Rural Engineering,
Food Research Institute,
Institute of Agrobiological Sciences,
Institute of Crop Science,
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Advanced Analysis Center,
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Kyushu Okinawa Agricultural Research Center,



Institute of Fruit Tree and Tea Science

NARO

Tea industry research division consists of four units.



Tea production areas in Japan



	Prefecture	annual yields	percentage of total
		Fresh weight (metric tons)	(%)
1	Shizuoka	144,400	40
2	Kagoshima	110,900	31
3	Mie	32,600	9
	…		…
	All Japan	357,800	100

*2015 report

In Japan, green tea is produced in Shizuoka the most, and Kagoshima ranks second.

The sum from both prefecture accounts for 70% of the total yields.

Our institute, NARO tea industry research division has two branches based in Shizuoka and Kagoshima prefectures.

The kinds of Japanese tea

★ Japanese green tea is basically "**Steamed unfermented tea**".



Camellia sinensis

茶

- **Unfermented tea（green tea）**
  - There are two methods as the polyphenol oxidase-inactivating processes (stopping fermentation).
  - **Steaming（蒸製）** ↑ This method can process many leaves (ex. 240kg) at one time.
    - **"Sencha"（煎茶）** is a needle-like shaped tea .
    - **"Bancha"（番茶）** is essentially **Sencha** made from tea leaves harvested during summer and fall.



    - **"Hojicha"（焙じ茶）** is roasted **Sencha** or **Bancha**.
    - **"Gyokuro"（玉露）** is **Sencha** made from leaves shaded from sunlight.



    - **"Matcha"（抹茶）** is **powdered green tea**, and is made from stone-mill ground **Tencha**.
    - Pan
  - Semi fermented tea
  - Fermented tea



Tencha is made from leaves **shaded from sunlight**.
Tencha is similar to **Gyokuro** but processed **without the kneading** prior to drying.
In general, **Tencha** is not sold to consumers.

The process for producing "Sencha"

Fresh harvested leaves are **steamed**, and they are then dried.
In particular, **Sencha** is dried while being **rolled and crumpled**.

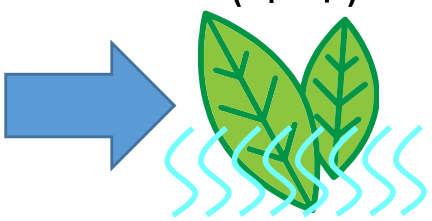
First

Steaming
(蒸)



The polyphenol oxidase is deactivated.

Cooling
(冷却)



The surface water is removed, and the steamed leaves are cooled.

Next **Drying (乾燥)**

The drying method consists of the following five processes.

Primary drying (粗揉)	Tea rolling (揉捻)	Secondary drying (中揉)	Final rolling (精揉)	Final drying (乾燥)
The steamed leaves are crumpled strongly with hot air (100℃). Leaf temperature is 35-36℃.	The water in leaves are kneaded at room temperature.	The leaves are crumpled with hot air. Leaf temperature is 35-36℃.	The leaves are shaped like needle. Leaf temperature is 35-36℃.	The final water content is about 5%.
About 50 min	About 40 min	About 30 min	About 45 min	About 30 min

*** In the case of machinery, total time is about 4 hrs.**

The method of tea refining



＝ 毛茶 in Taiwan？

Final dried tea leaves are called 荒茶 =unrefined tea.

The unrefined tea contains powder, stems, and some big leaves .





Powder, buds, and stems are separated by sieving.

separating process by sieving

buds

stems

Bud tea

Sencha

Stem tea







Refined tea is **Sencha**.

Buds are “**Bud tea**”= 芽茶.
Stems are “**Stem tea**”= 茎茶.
These are by-products of **Sencha**.

Functional ingredients in tea

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★ Catechins (EGCG, EGC, ECG, EC etc.)

★ Caffeine

★ Theanine

★ Quercetin

★ GABA (γ-amino butyric acid)

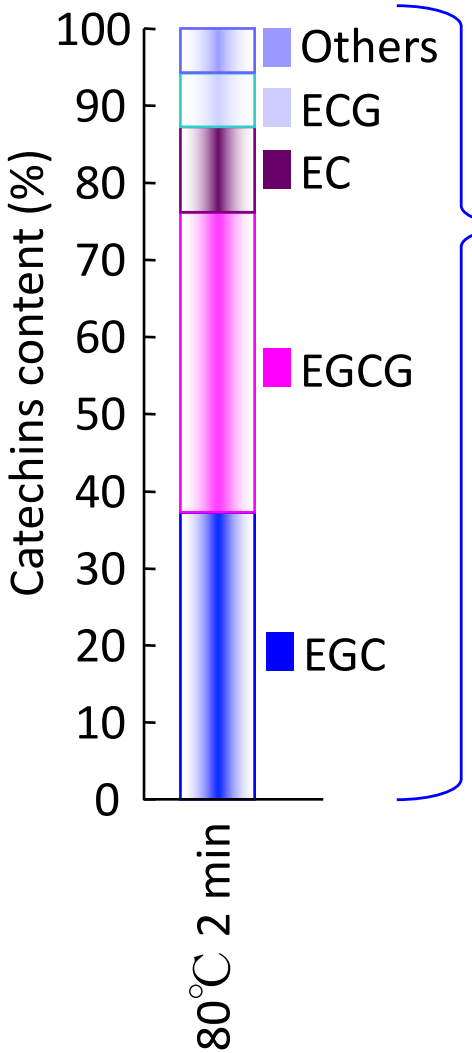


These are major functional ingredients in tea. But, these ingredients are influenced by various conditions.

Catechins

The difference between EGCG and EGC is presence or absence of a gallate moiety.

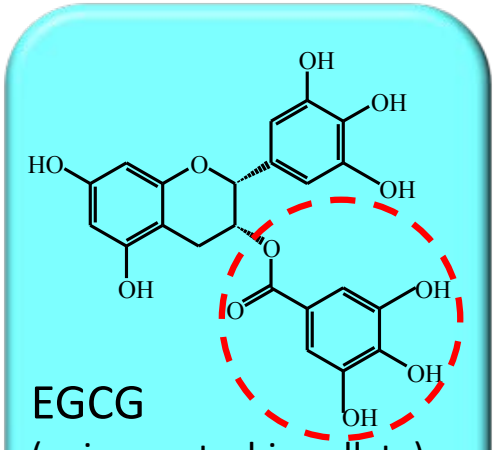
In hot water infusion





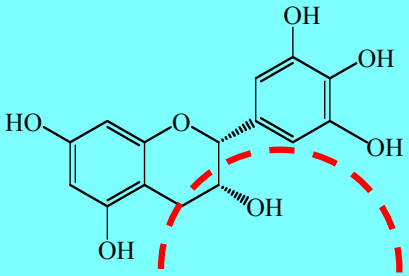
About 70% is EGCG and EGC.

Gallate type



EGCG
(epigarocatechin gallate)

Non-Gallate type



EGC
(epigarocatechin)

Anti-oxidant & Anti-bacterial effect

Both catechins have these effects.

Anti-tumor

Anti-inflammation
(Immune-suppressing)

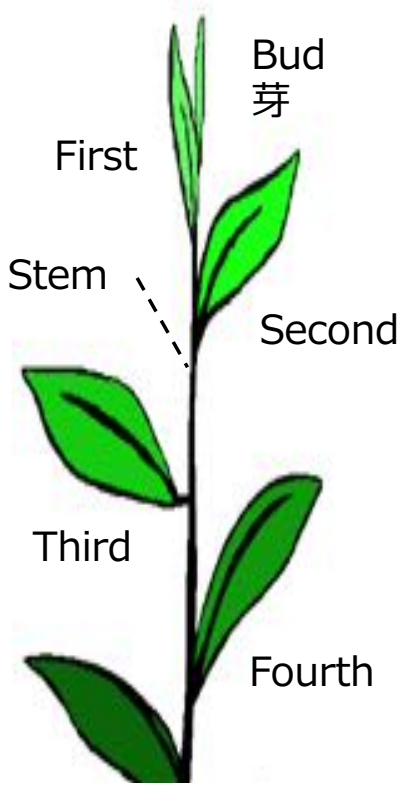
These effects are typical for EGCG.

Immune-enhancing

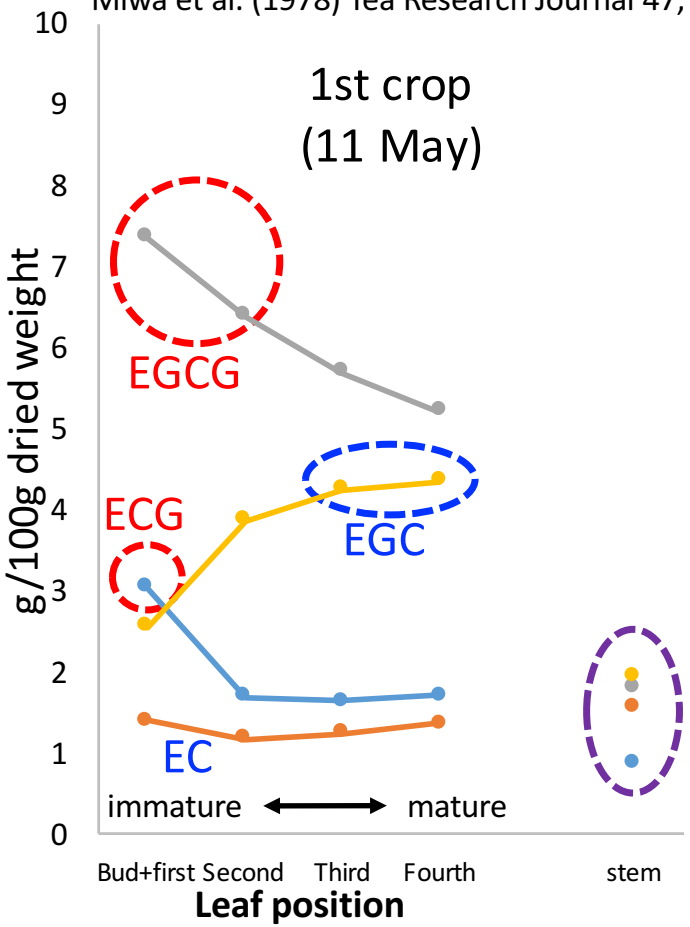
But, this effect is offset by EGCG.

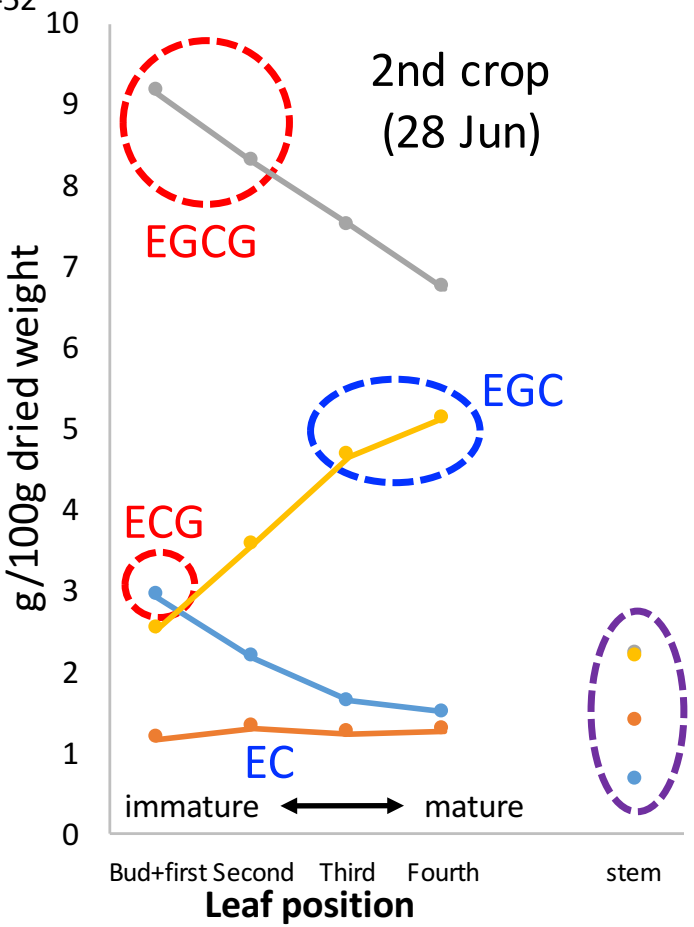
Maturity × Catechins ①

Catechins content changes with **leaf position**.



Miwa et al. (1978) Tea Research Journal 47, 48-52





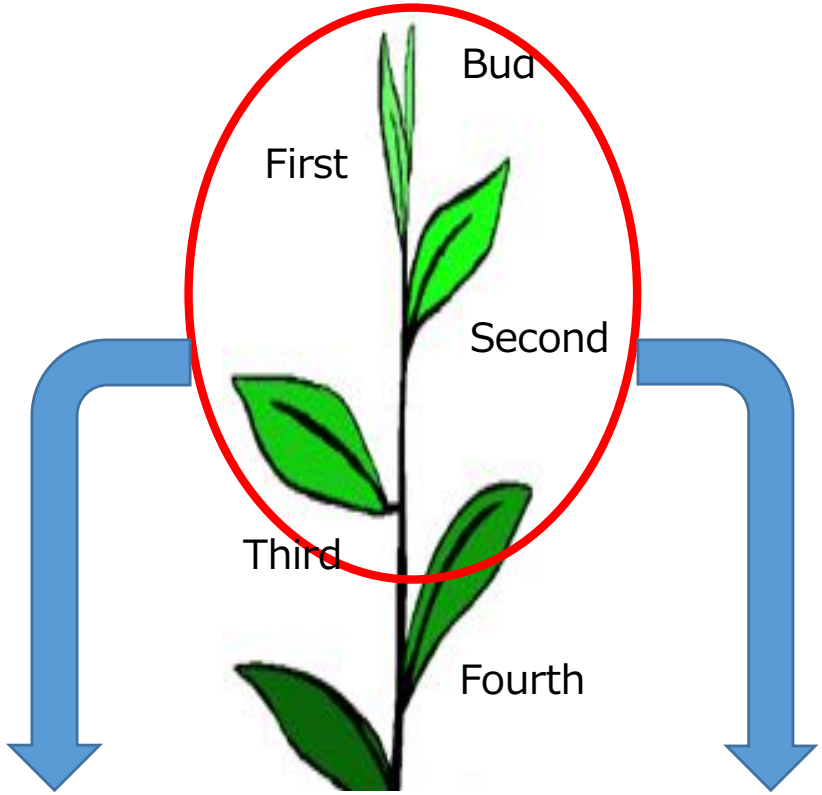
Gallate-type catechins are rich in immature upper leaves.

Non gallate-type catechins, especially EGC is rich in mature lower leaves.

Catechins are only found at low levels in stems, and are higher in the second crop than in the first crop.

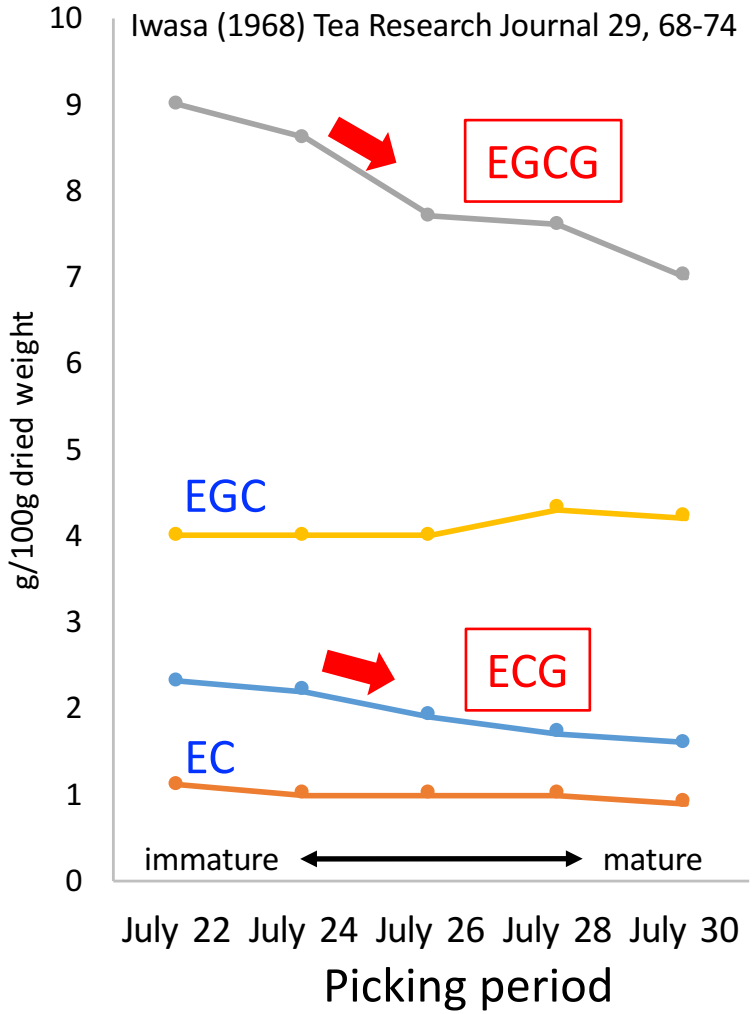
Maturity × Catechins ②

Catechins content changes with **picking period**.



Early picking?

Late picking?

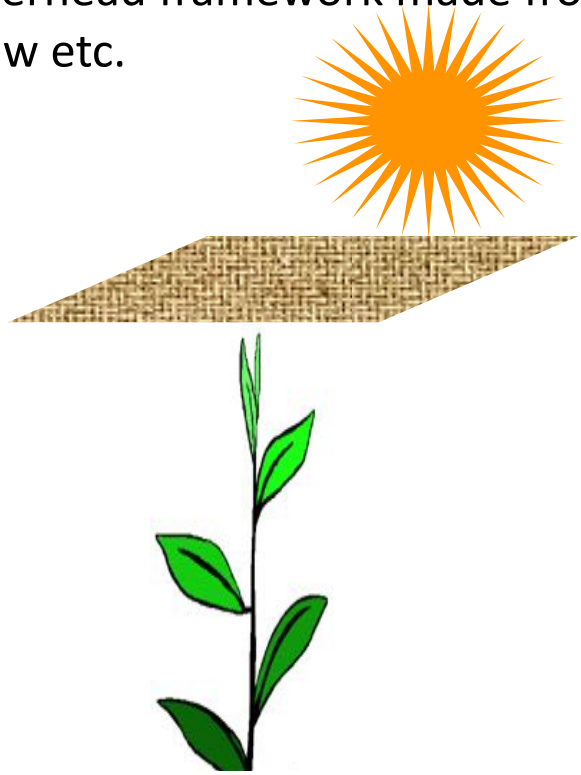


Gallate-type catechins are decreased by a delay of picking.

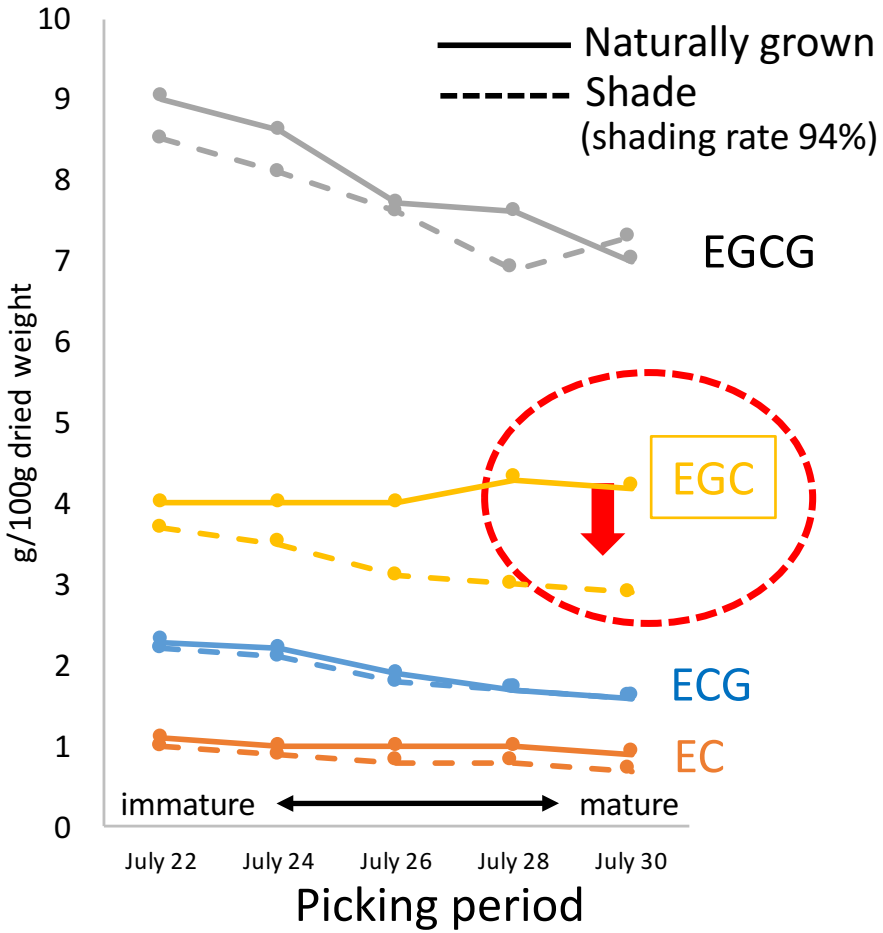
Shading treatment × Catechins

Catechins content changes with **sunlight**.

With regard to Japanese **“Matcha” (抹茶)** and **“Gyokuro” (玉露),** the tea bushes are completely shaded for about 20 days using a large overhead framework made from reeds and rice straw etc.



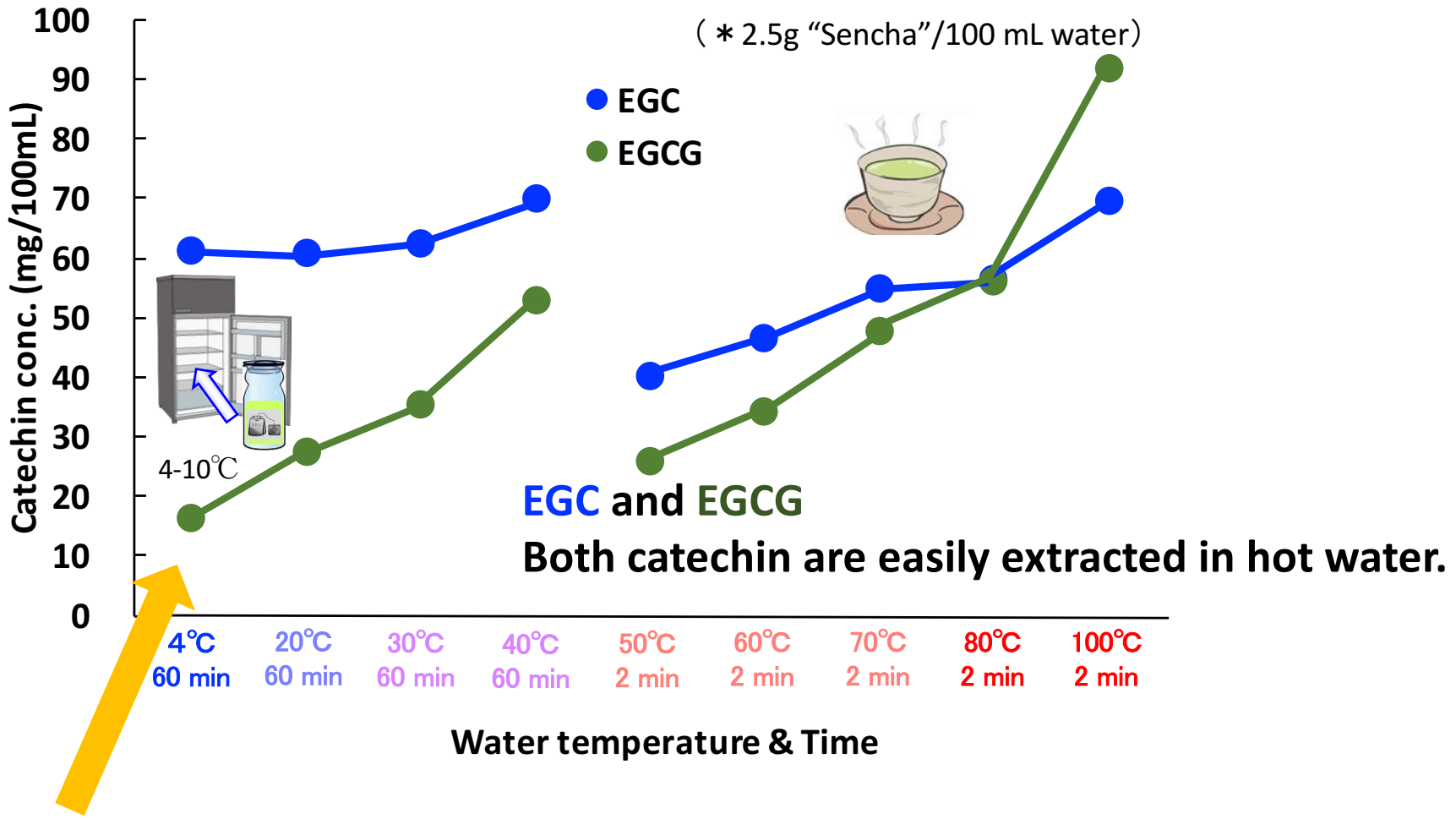
Iwasa (1968) Tea Research Journal 29, 68-74



Shading treatment decreases non-gallate-type catechin, especially EGC. EGCG is not affected by shading treatment.

Water Temperature × Catechins

Catechins content changes with **water temperature.**



But EGCG is difficult to extract in cold water.

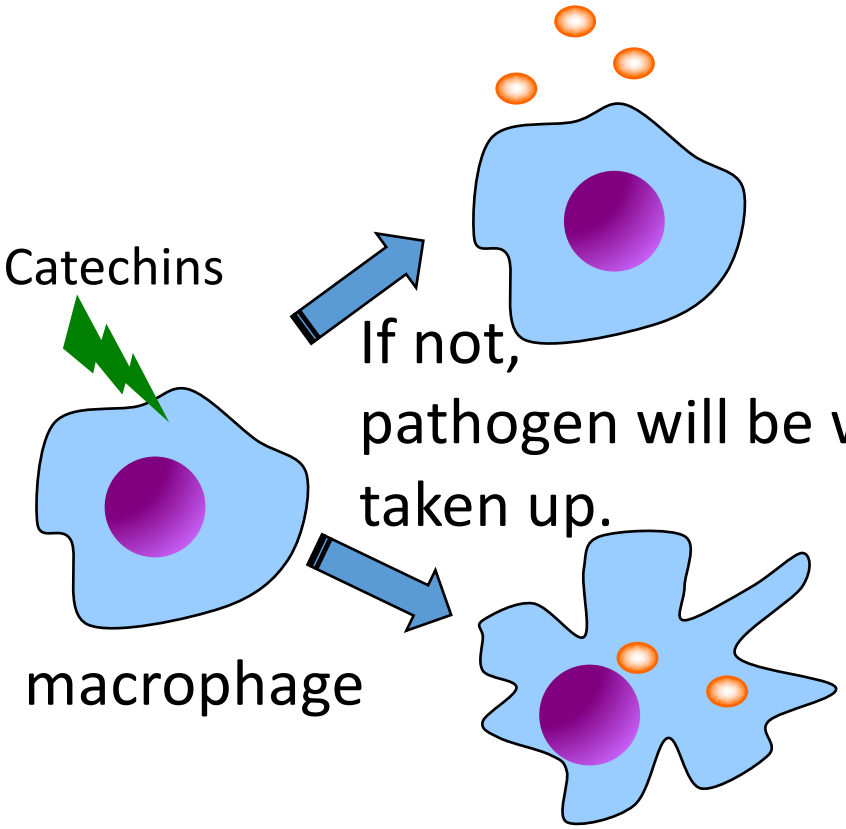
On the other hand, EGC is easily extracted in cold water.

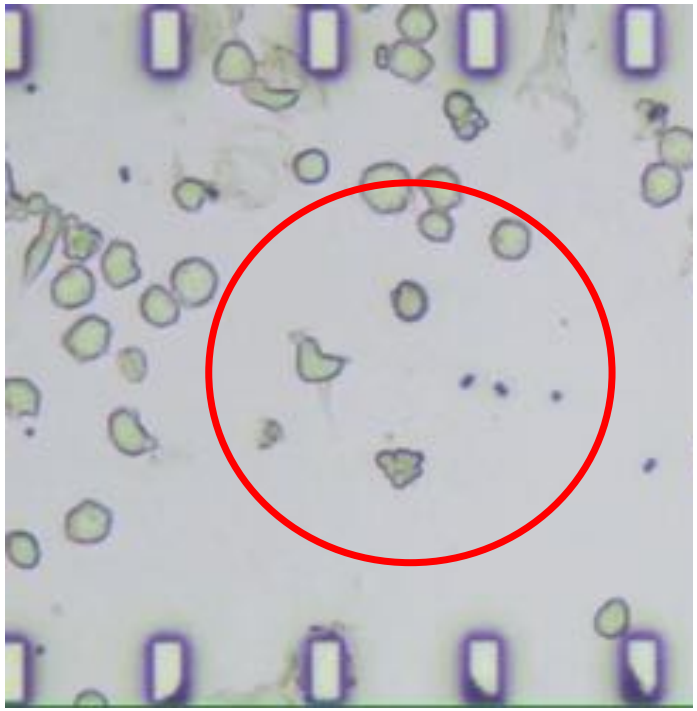
The immune enhancing effect of EGC

As explained in advance, **EGC** has the immune enhancing effect.

EGC has a good effect on macrophage phagocytosis.

Macrophage is one of the immune cells.





fast-forward image

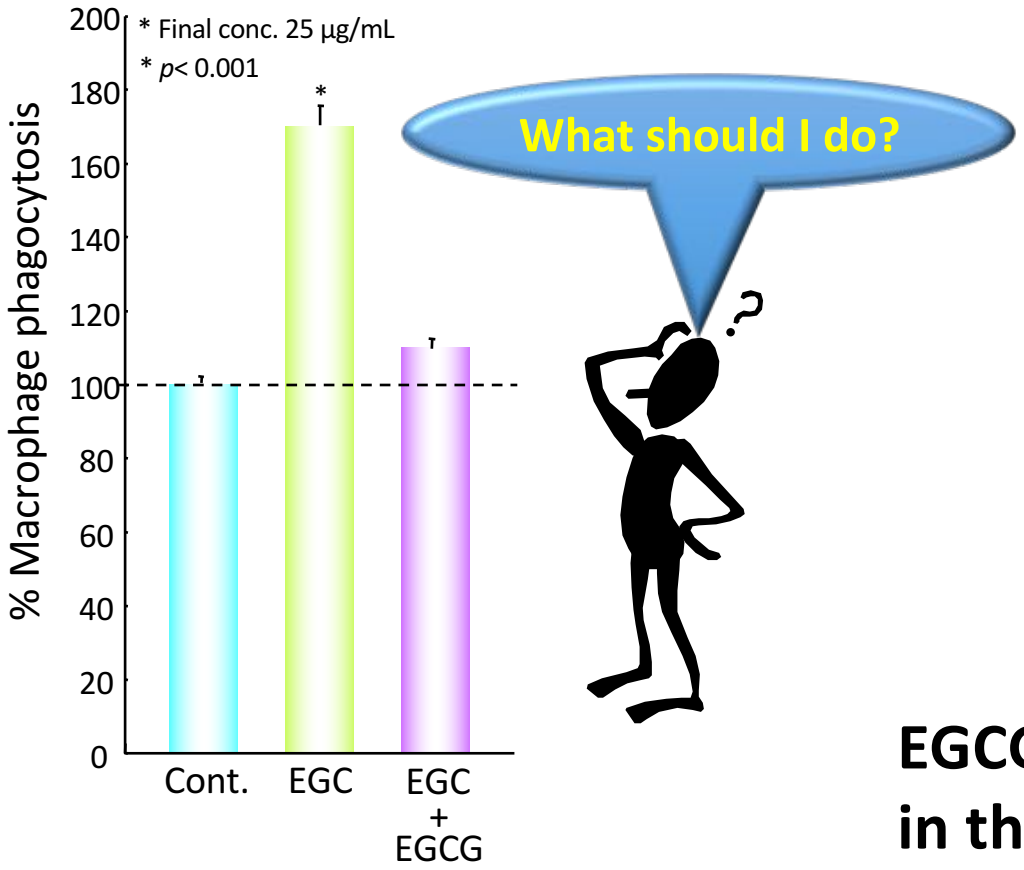
If macrophage is activated by catachins,
pathogen will be actively taken up into cells.

The immune enhancing effect of EGC

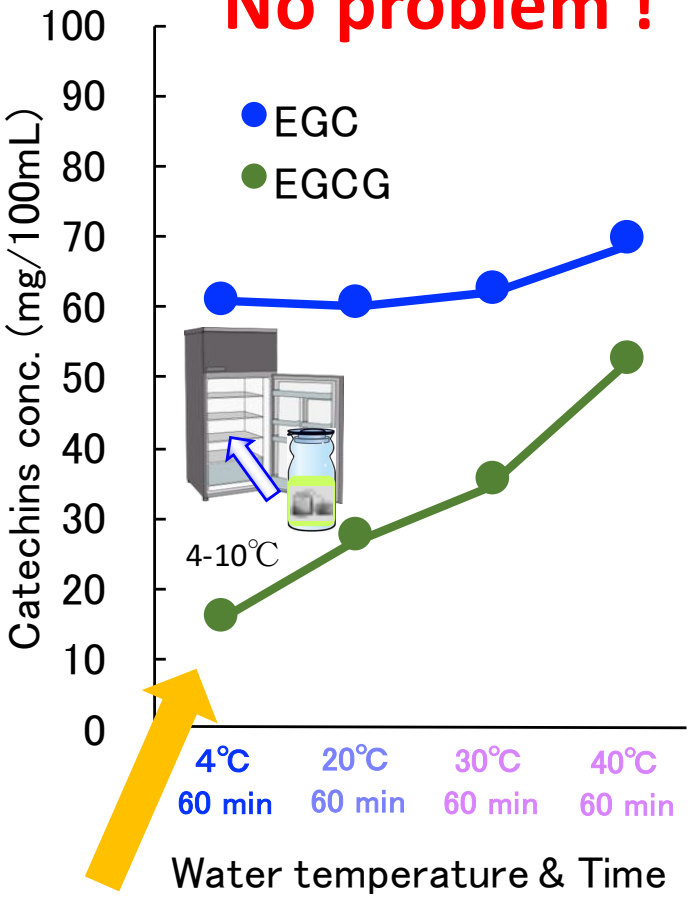
But, **the effect of EGC is cancelled out by EGCG.**

EGC activates the phagocytosis of macrophages.

But, the mixture of EGC and EGCG does not have the activity.



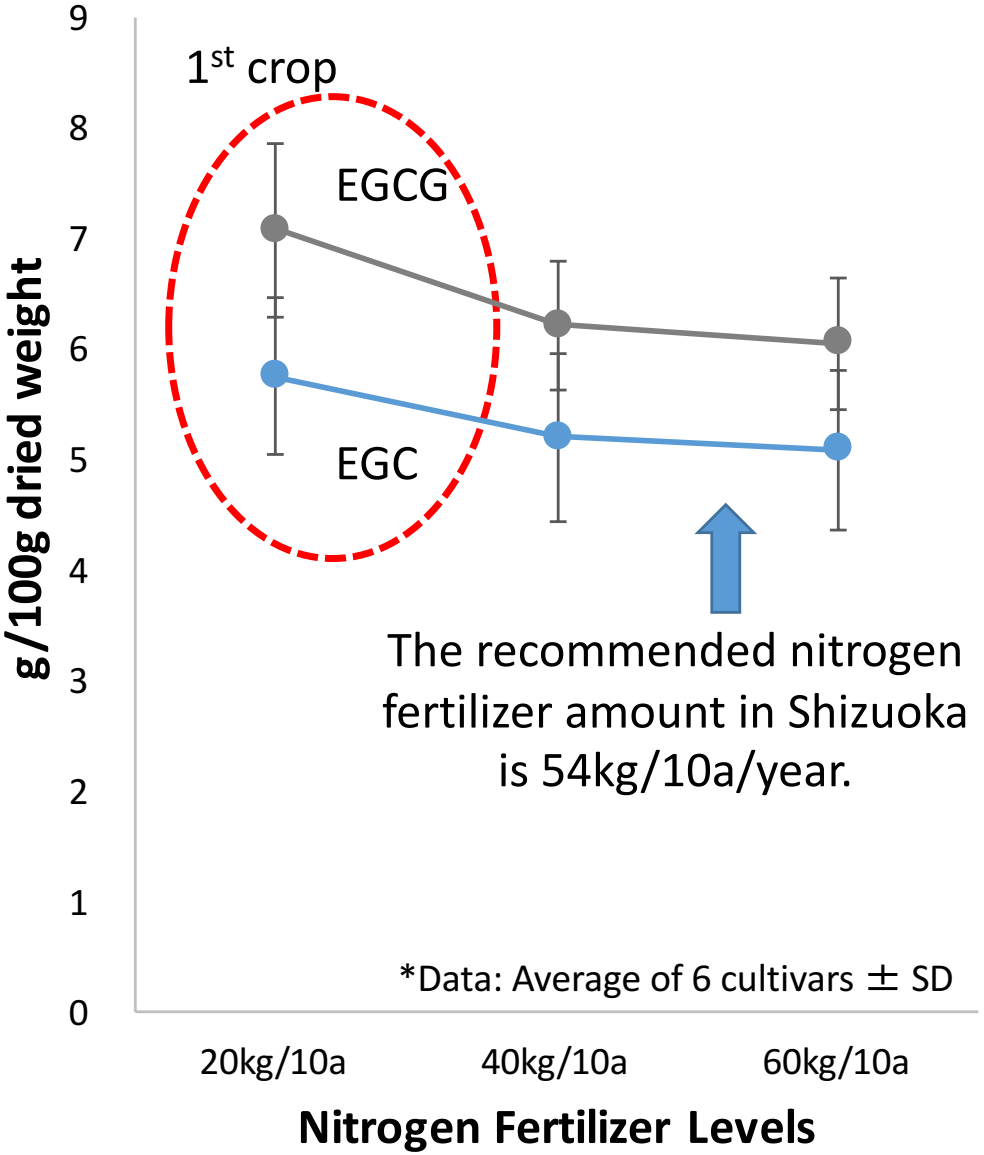
No problem !



EGCG is found only at low levels in the cold infusion.

Fertilizer × Catechins

Catechins content changes with **nitrogen fertilizer amount**.



Matsunaga et al. (2009) Tea Research Journal 108, 19-27

Apparent increases in the concentrations of catechins were observed with 20kgN/10a/year.
The cause is unknown.

But, the recommended nitrogen fertilizer amount is 54kgN/10a/year in Shizuoka.

It is reported that a level of 27kgN/10a/year negatively affected growth and yield.

Cultivars × Catechins

There are about 100 tea cultivars in Japan. But, **“Yabukita” 藪北 is a main tea cultivar, which occupies 80% of tea field**. Some cultivars have more ingredients than “Yabukita”.



Cultivars × Catechins

Differences in cultivars affect the catechin content.

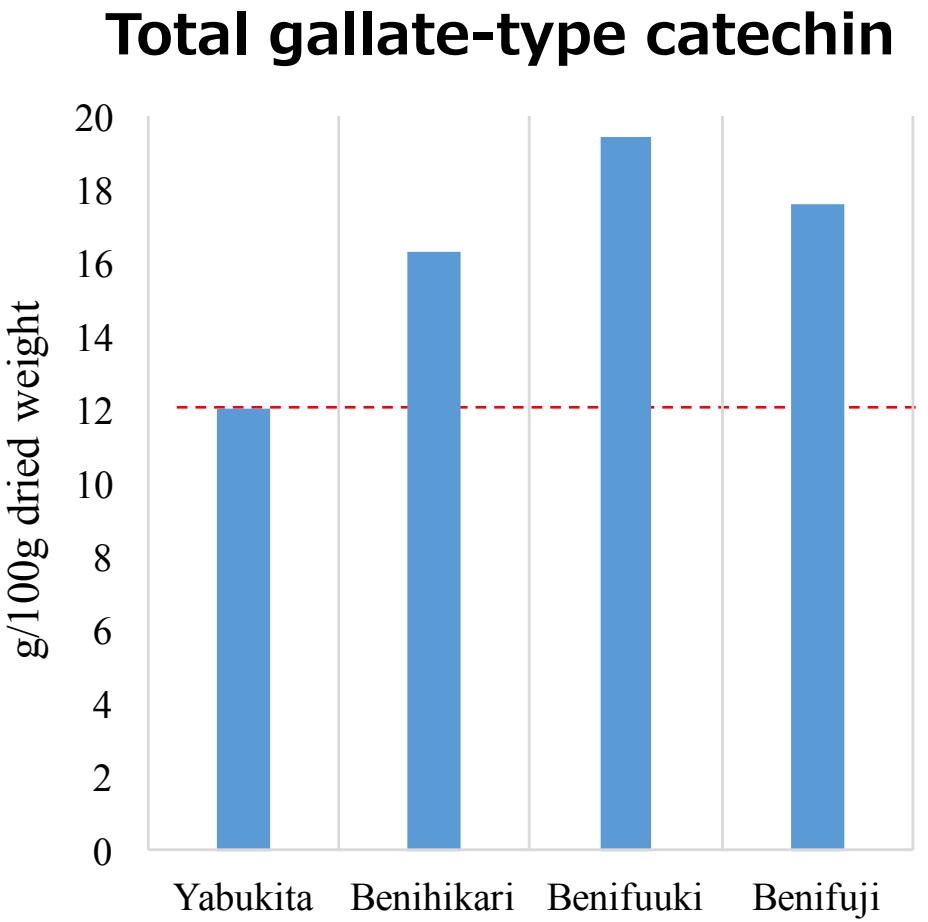
For example, “**Benifuuki**” contains higher amounts of gallate-type catechins compared with “Yabukita”.

EGCG rich cultivar

“Benifuuki” 紅富貴

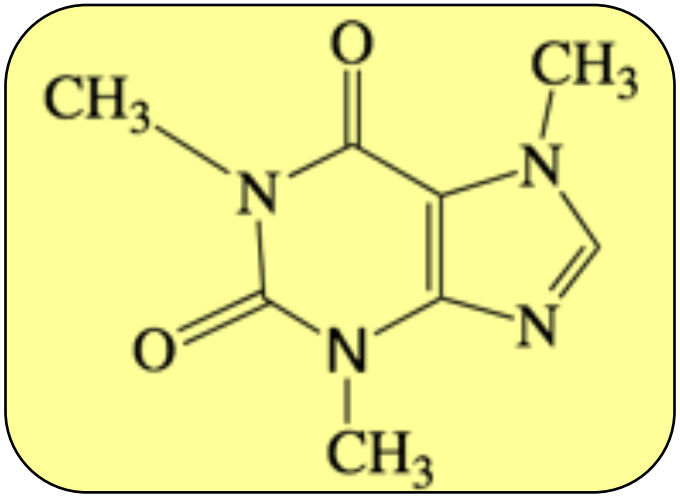
“Benifuuki” was bred as cultivar for making black tea, but currently it is also made to green tea because of its anti-allergic EGCG3"Me (methylated catechin) of content.

In general, the cultivars for making black tea such as “Benifuuki”, “Benihikari”, and “Benifuji” are rich in gallate-type catechins compared with “Yabukita”.



Maeda-Yamamoto M et al. (2001) 48: 64–68

Caffeine



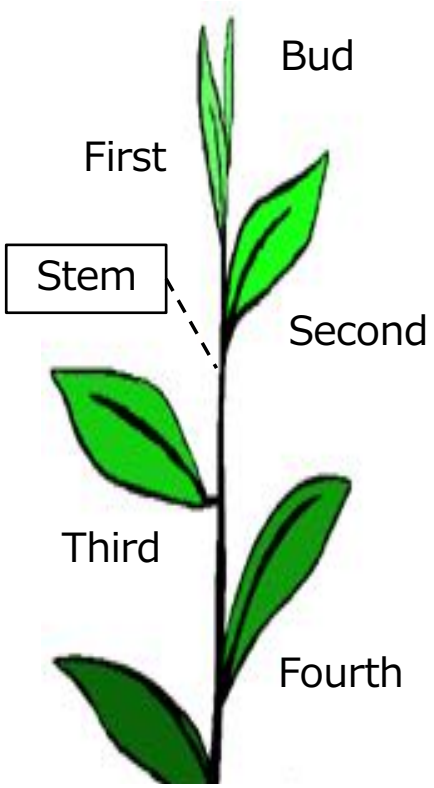


★ Caffeine is best-known psychostimulant, and it disturbs sleep.

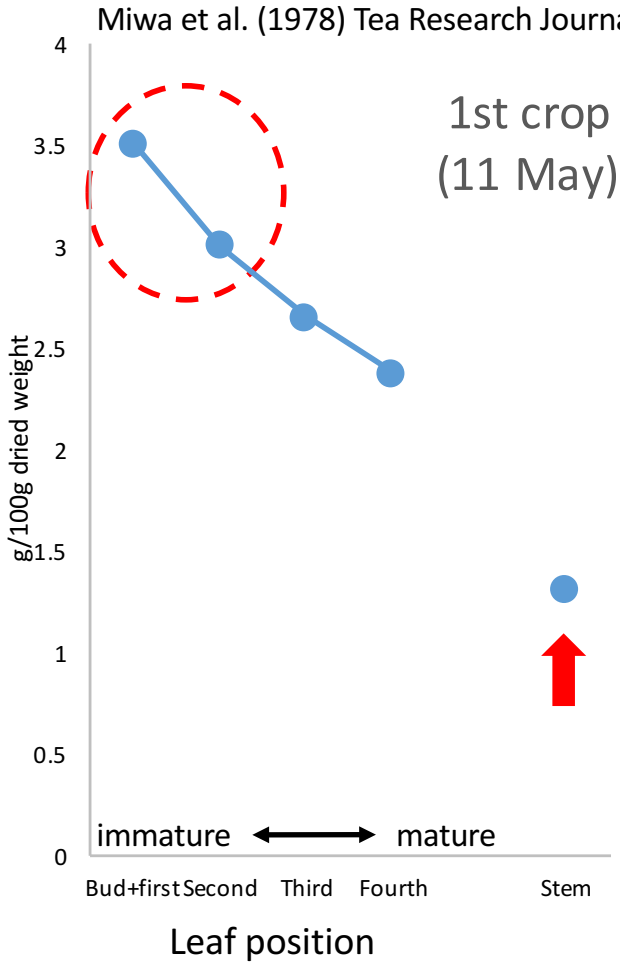
★ Although some consumers want to have caffeine present, others want to cut down on it.

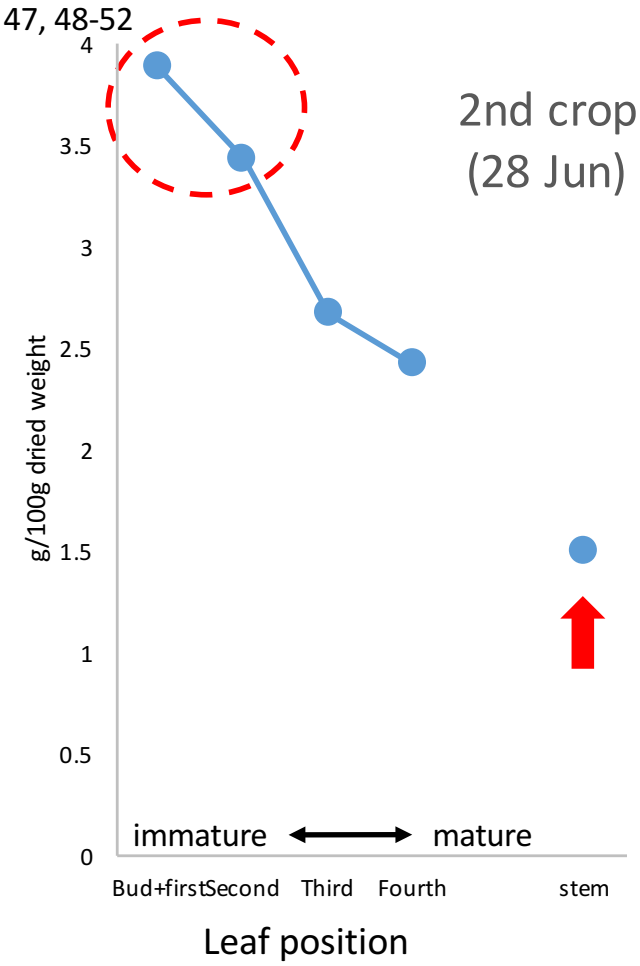
Maturity × Caffeine ①

Caffeine content changes with **leaf position**.



Miwa et al. (1978) Tea Research Journal 47, 48-52

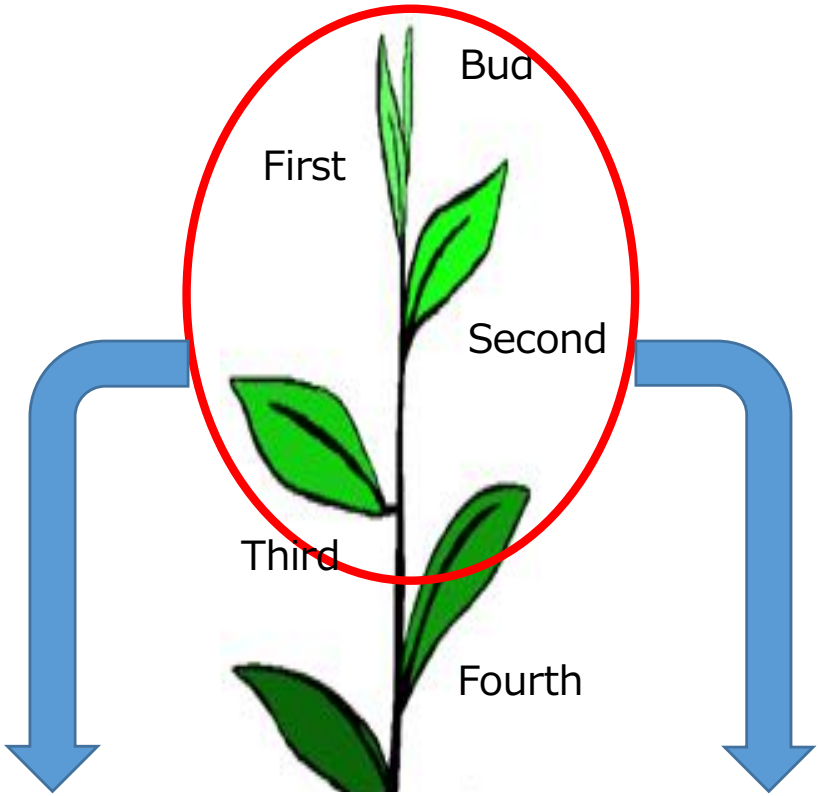




Caffeine is rich in immature upper leaves.
But, its levels are poor in stems.

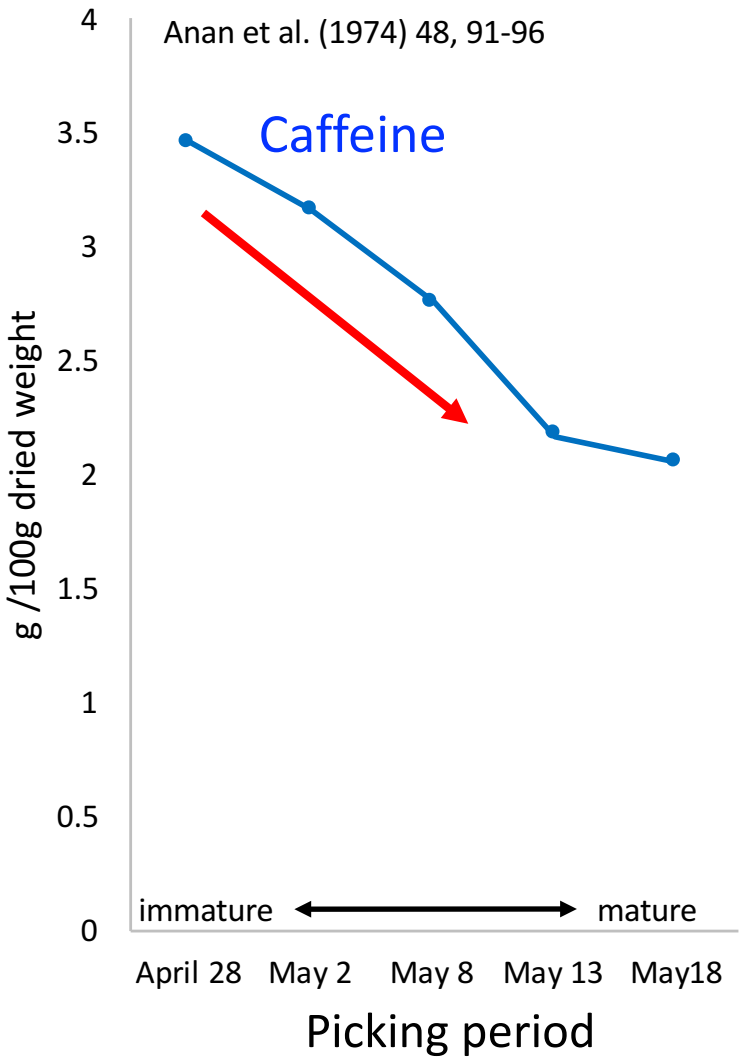
Maturity × Caffeine ②

Caffeine content changes with **picking period**.



Early picking?

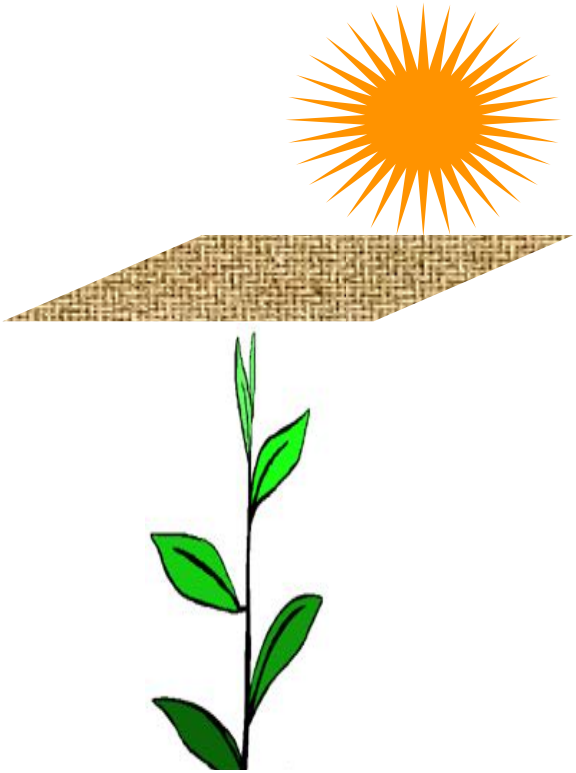
Late picking?



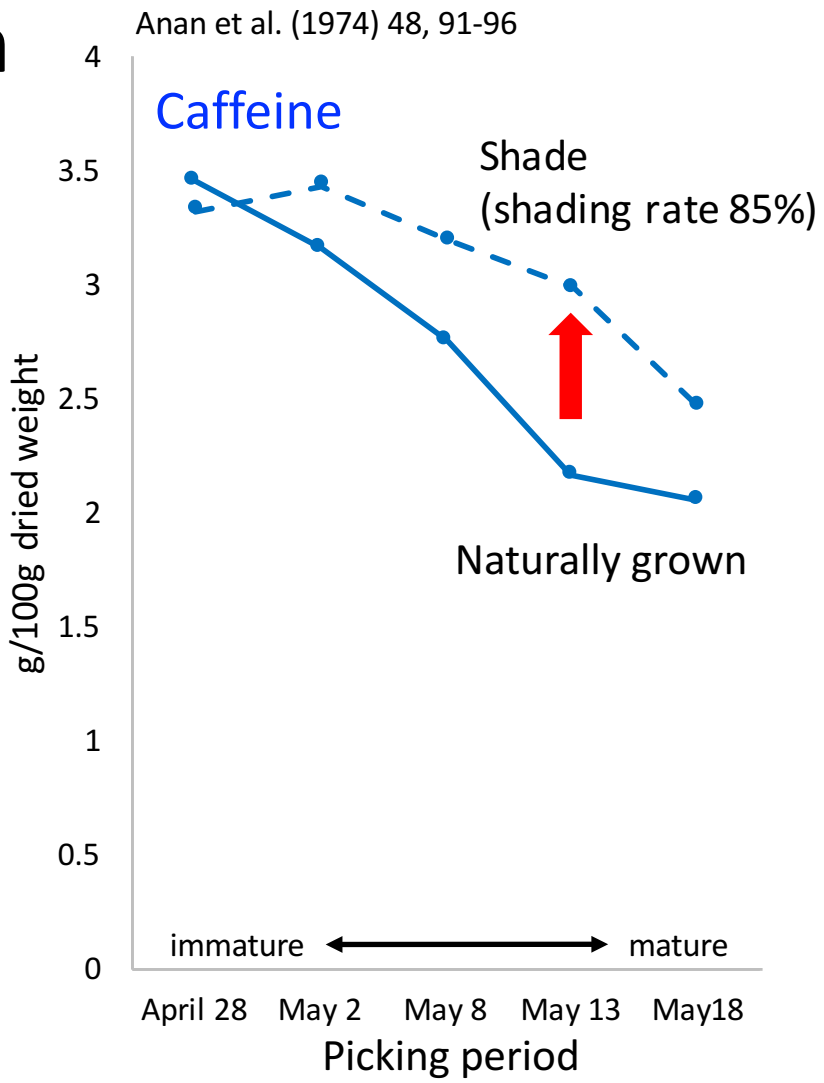
Caffeine levels are decreased by a delay of picking.

Shading treatment × Caffeine

Caffeine content changes with **sunlight**.



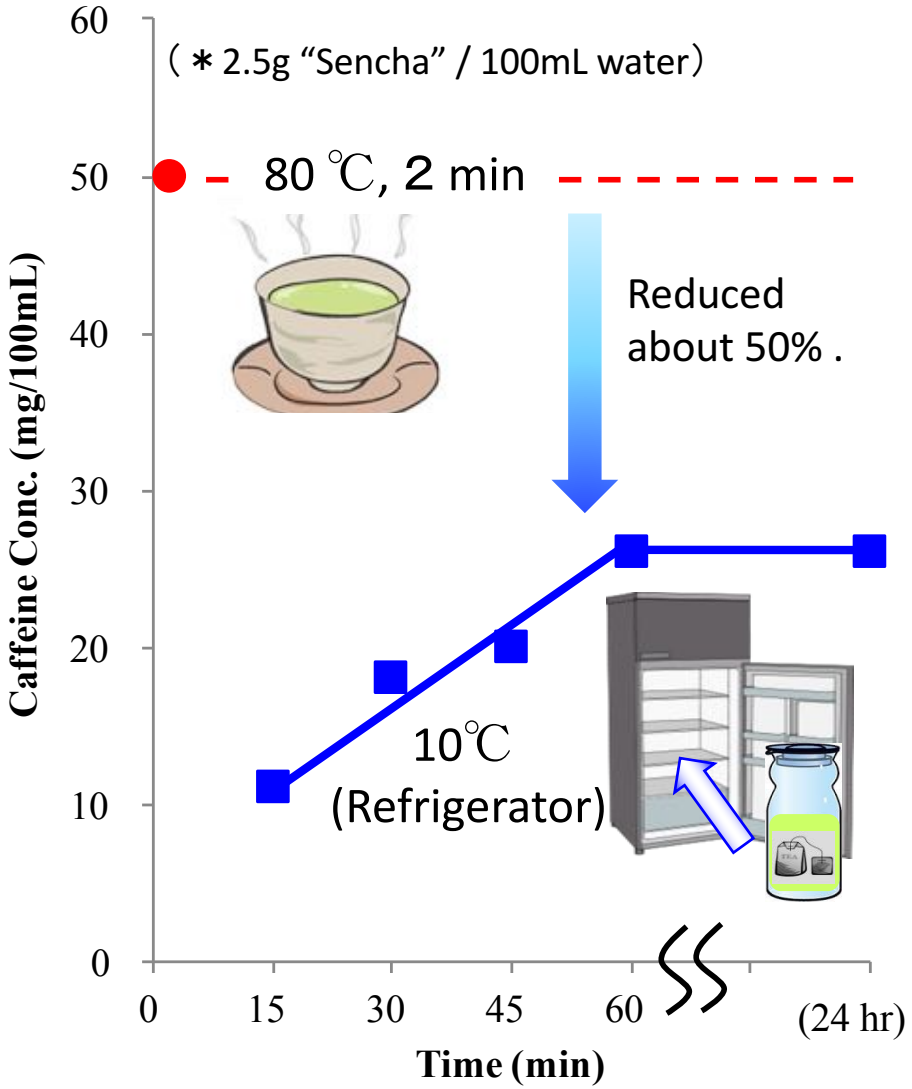
Anan et al. (1974) 48, 91-96



Shading treatment prevents the decrease in caffeine associated with maturation .

Water Temperature × Caffeine

Caffeine is difficult to extract in cold water (under 10℃).



When cold water (under 10℃) was used, caffeine level after an infusion for 1 hr was reduced to about 50% of that with the hot water infusion (80℃, 2 min).

Further extraction did not affect the concentration of caffeine.

Moreover, when iced water (0.5℃) was used, the caffeine in the infusion was under 20% of that in a hot water infusion (80℃, 2 min).

Caffeine levels can be reduced in manufacturing process.

This machine is able to reduce a caffeine content by **about 50%** by heated water shower (95℃) onto fresh tea leaves for more than 90 seconds.

This machine has been sold in TERADA SEISAKUSHO CO.,LTD.

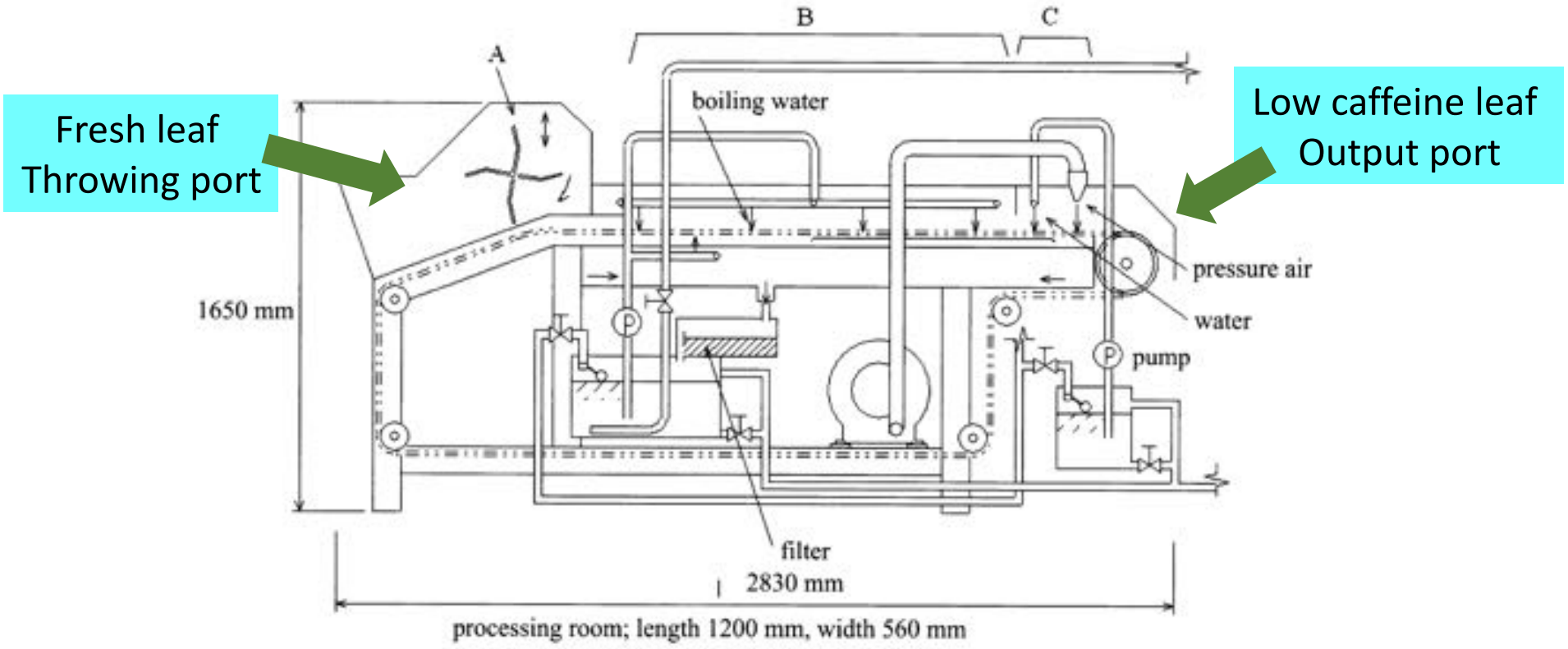


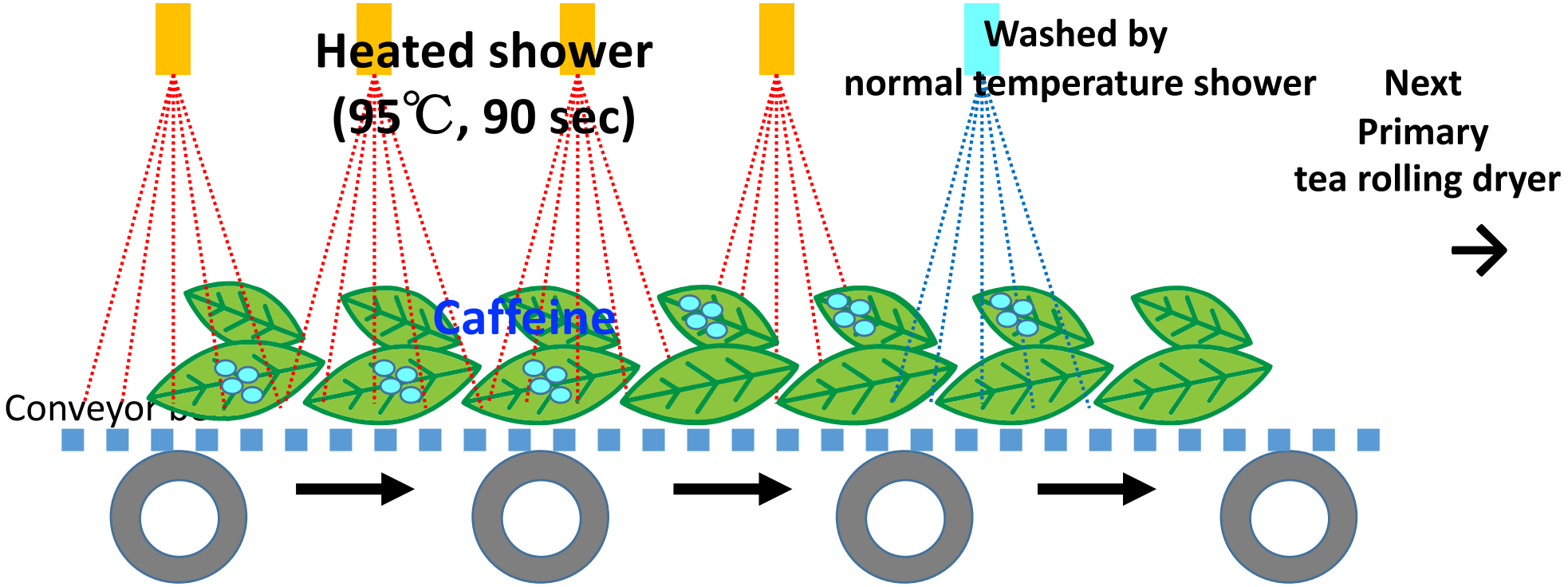
Fig. 1 Figure of low caffeine processing machine LCT160001 (50K).

Journal of the Japanese Society for Food Science and Technology 8(3) 109-116 (2007)

Low caffeine processing machine

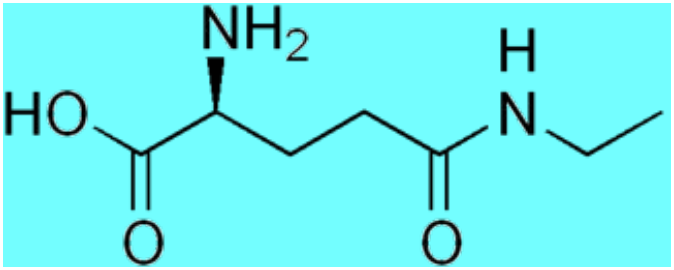
To put it simply,

1. “Steam processing” changes to a “heated water shower”.
2. **Caffeine floats** on the leaf surface by the heated shower.
3. The caffeine is **washed off** by a normal temperature shower.
4. The drying process is same as “Sencha”.



This treatment does not affect the content of other ingredients such as catechins and amino acids.

Theanine



★ Theanine is an amino acid analogue of L-glutamic acid.

★ It is well-known that oral intake of L-theanine causes a feeling of relaxation and prevents psychosocial stress .

Cultivar × Theanine

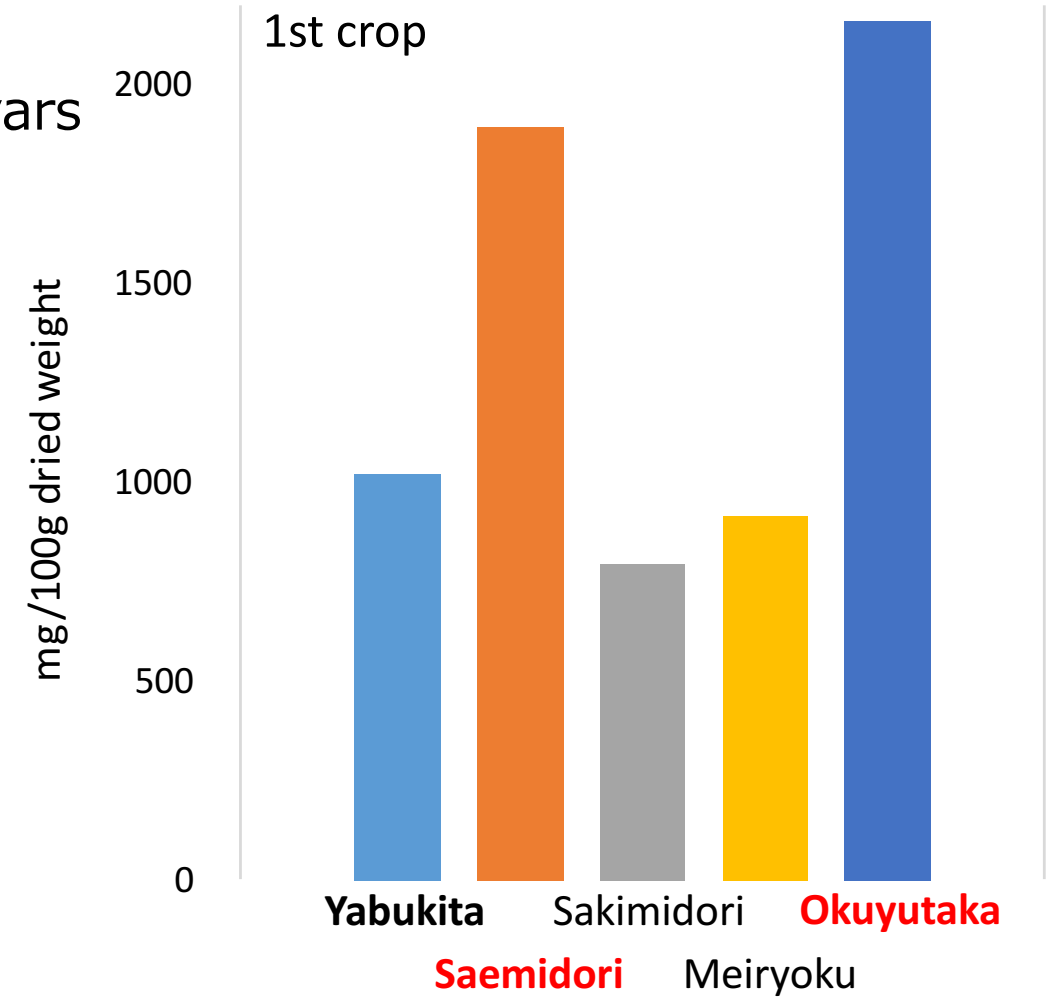
Differences in cultivars affect the theanine content.

For example, Japanese tea cultivars “Saemidori” and “Okuyutaka” are known as theanine-rich cultivars.

Theanine rich cultivar

“Saemidori”
“Okuyutaka”
“Harumidori”
.
.
.

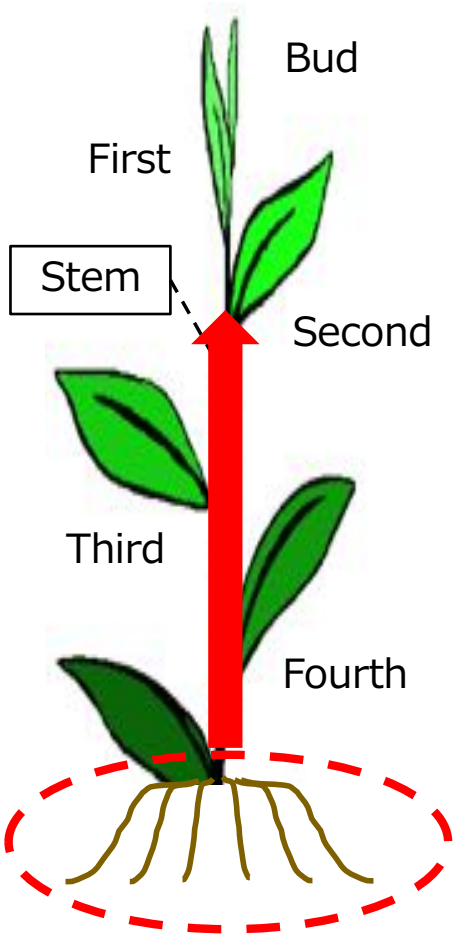
Matsunaga et al. (2005) Tea Research Journal 100(suppl.) 24-25



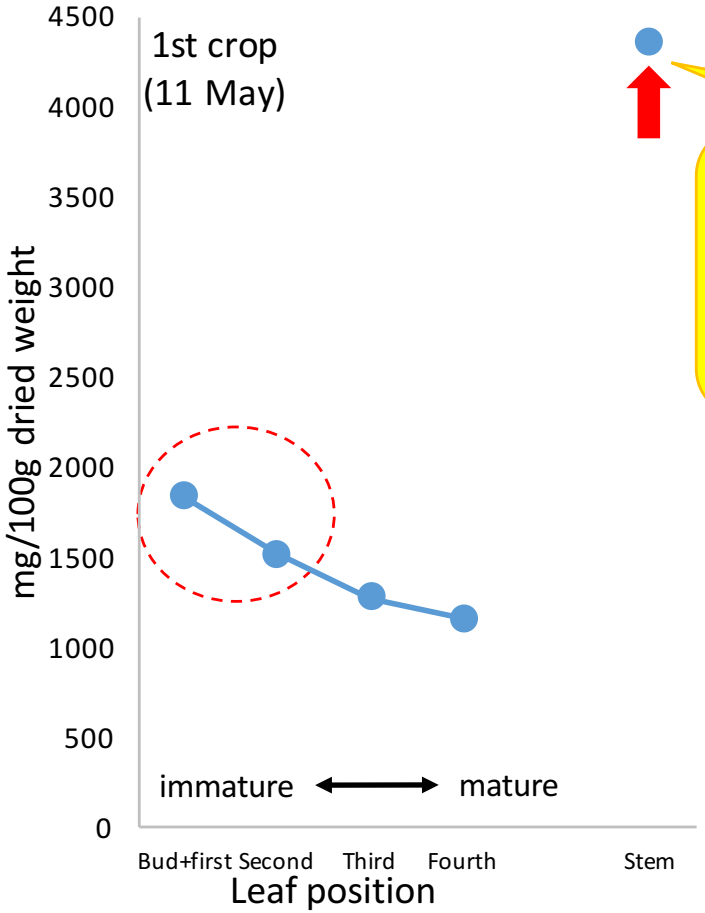
The theanine rich cultivars are often processed as “Gyokuro” in Japan.

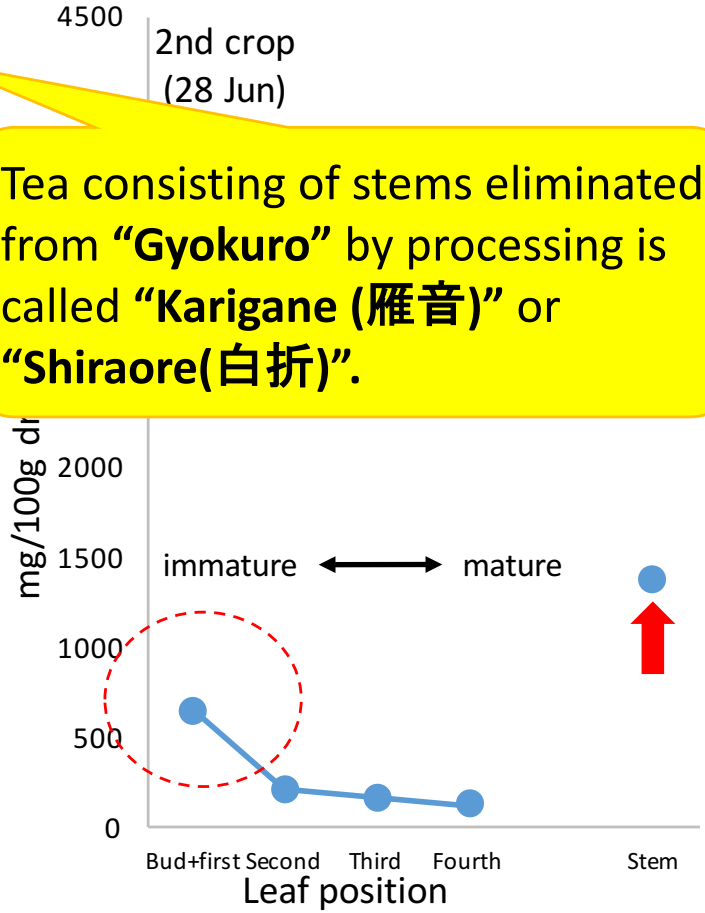
Maturity × Theanine ①

Theanine content changes with **leaf position**.



Miwa et al. (1978) Tea Research Journal 47, 48-52





Tea consisting of stems eliminated from **“Gyokuro”** by processing is called **“Karigane (雁音)”** or **“Shiraore(白折)”.**

Theanine is rich in immature upper leaves.

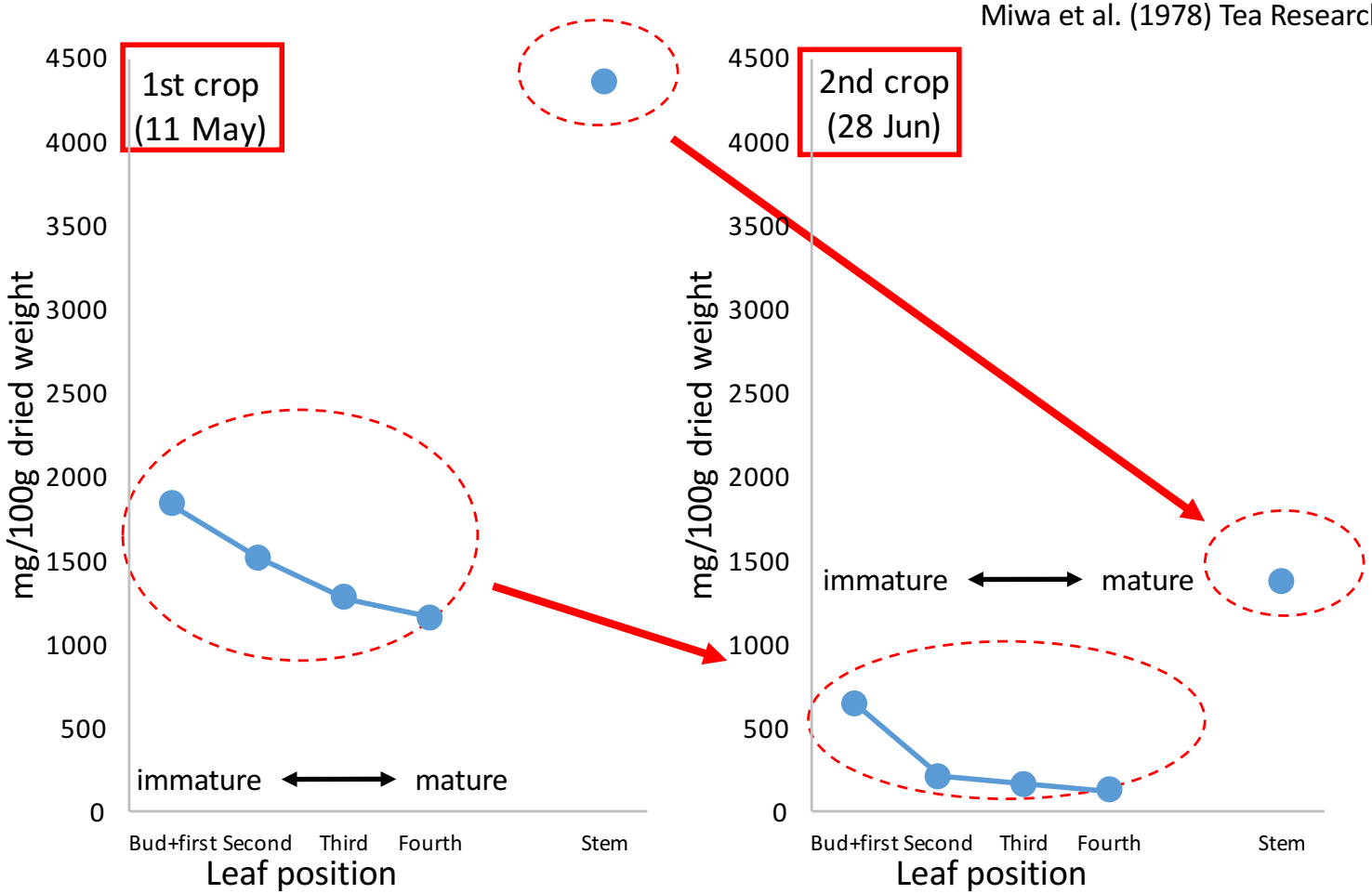
and, theanine is rich in stem unlike other ingredients.

Theanine is produced in the roots and is sent to the leaves.

Maturity × Theanine ②

Theanine content changes with **picking period**.

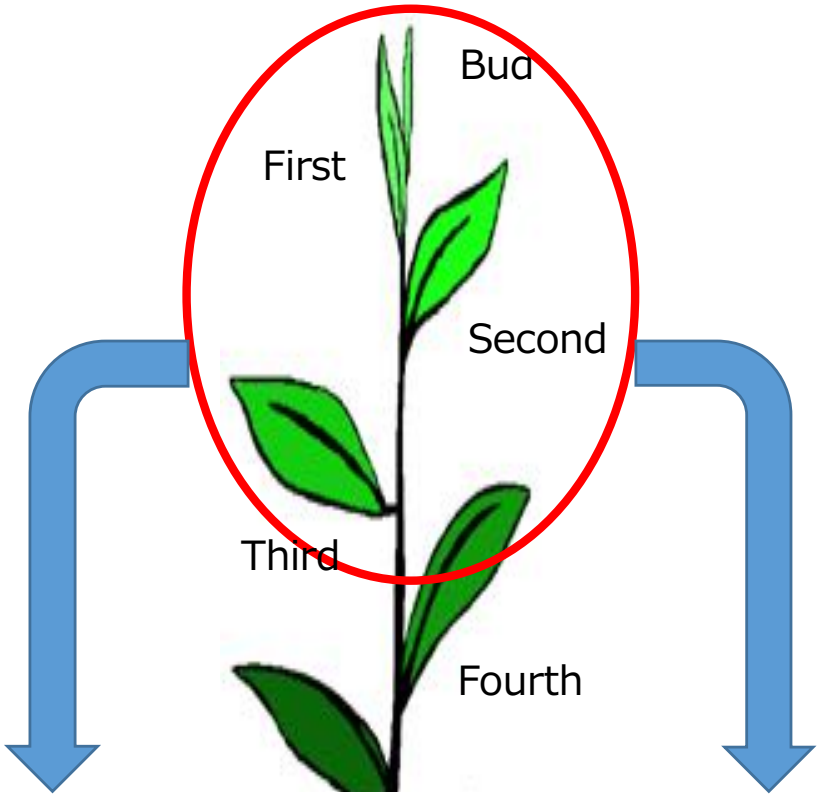
Miwa et al. (1978) Tea Research Journal 47, 48-52



Theanine content in the first crop is higher than that in the second crop.

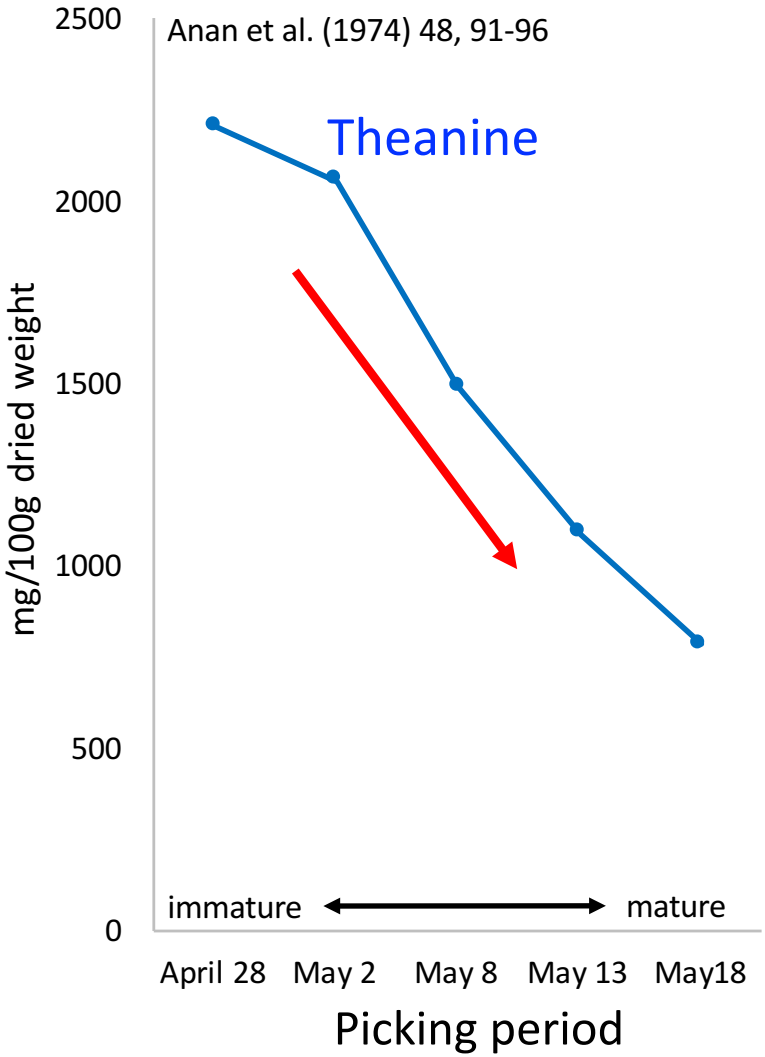
Maturity × Theanine ②

Theanine content changes with **picking period**.



Early picking?

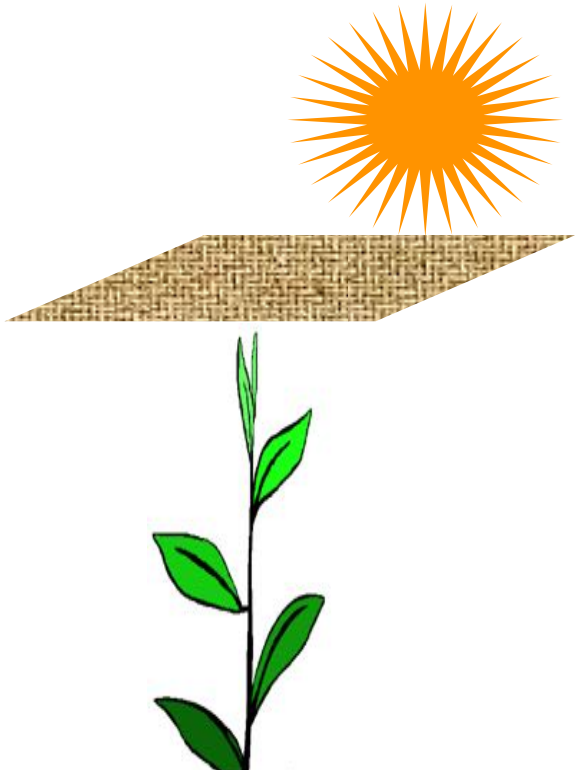
Late picking?



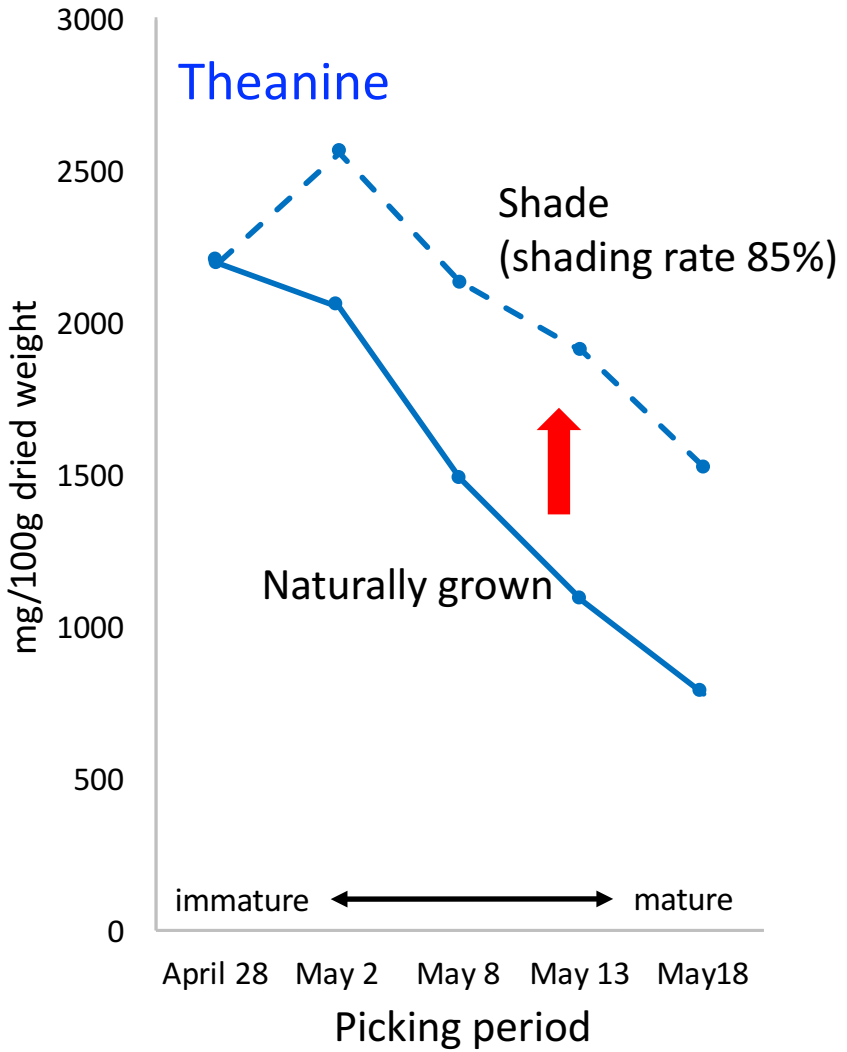
Theanine levels are decreased by a delay of picking.

Shading treatment × Theanine

Theanine content changes with **sunlight**.



Anan et al. (1974) 48, 91-96



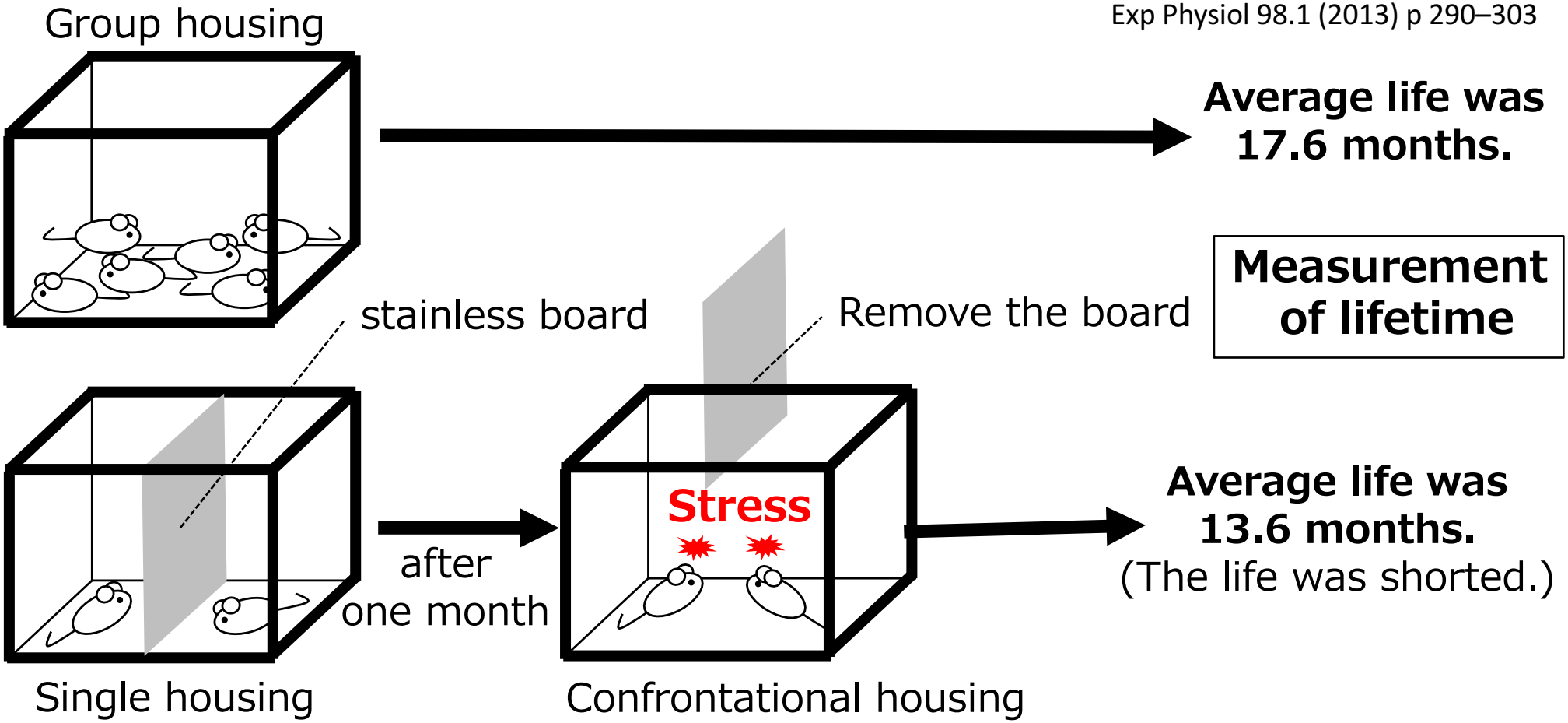
Shading treatment prevents the decrease in theanine associated with maturation.

Psychosocial stress-reducing effect

Psychosocial stress is induced by human relationship.

This stress also reduces the lifetime of mice.

Exp Physiol 98.1 (2013) p 290–303



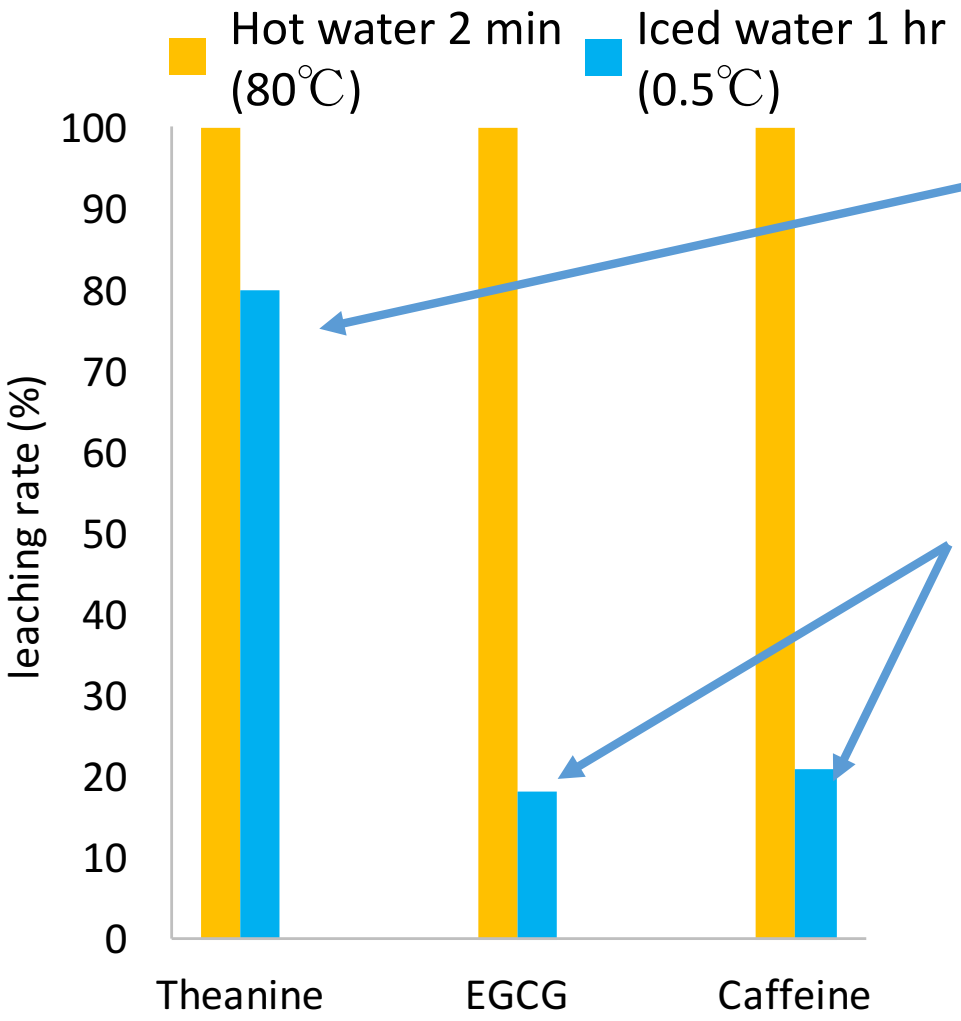
But, in confrontational housing the theanine-intake group's average life was 17.9 months.

Caffeine and EGCG reduce the effect.

Recently, it was reported that
the psychosocial stress-reducing effect by theanine
was cancelled by caffeine and EGCG.



Water Temperature × Theanine



The leaching rate at 80℃ for 2 min was set at 100%.

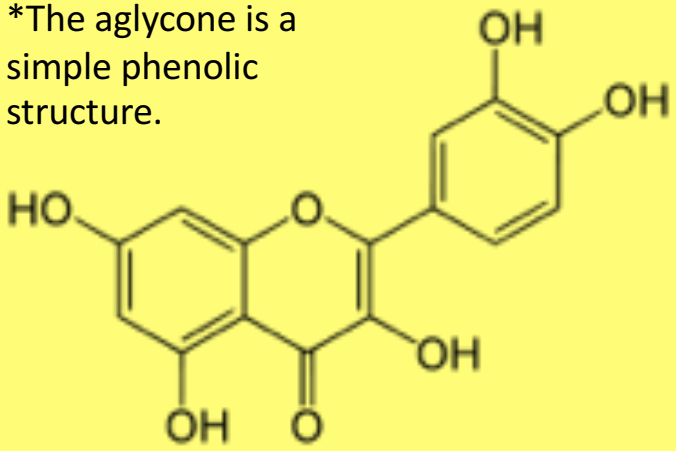
Theanine is relatively easily extracted in iced water (0.5℃).

On the other hand, EGCG and caffeine are difficult to extract in iced water.

EGCG and caffeine levels can be decreased. Consequently, their interference effect is reduced.

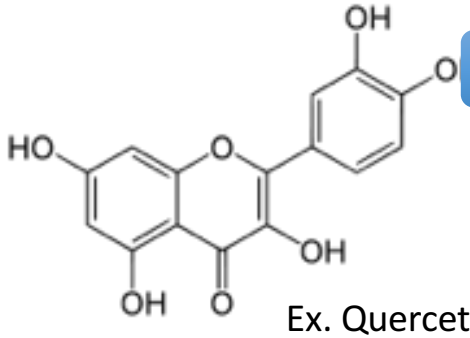
Quercetin

*The aglycone is a simple phenolic structure.



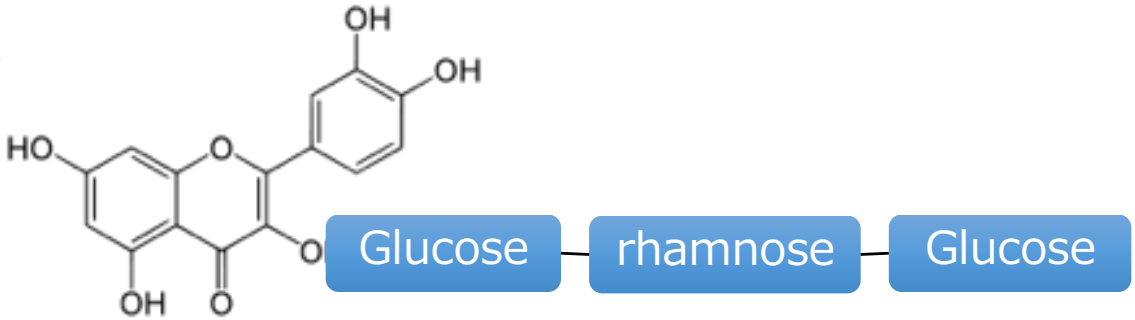
★ Quercetin is one of the most well-known flavonols and generally present in a glycoside form, and it has been reported to have anti-oxidation and cholesterol-lowering effects.

★ Onion quercetin is well-known.



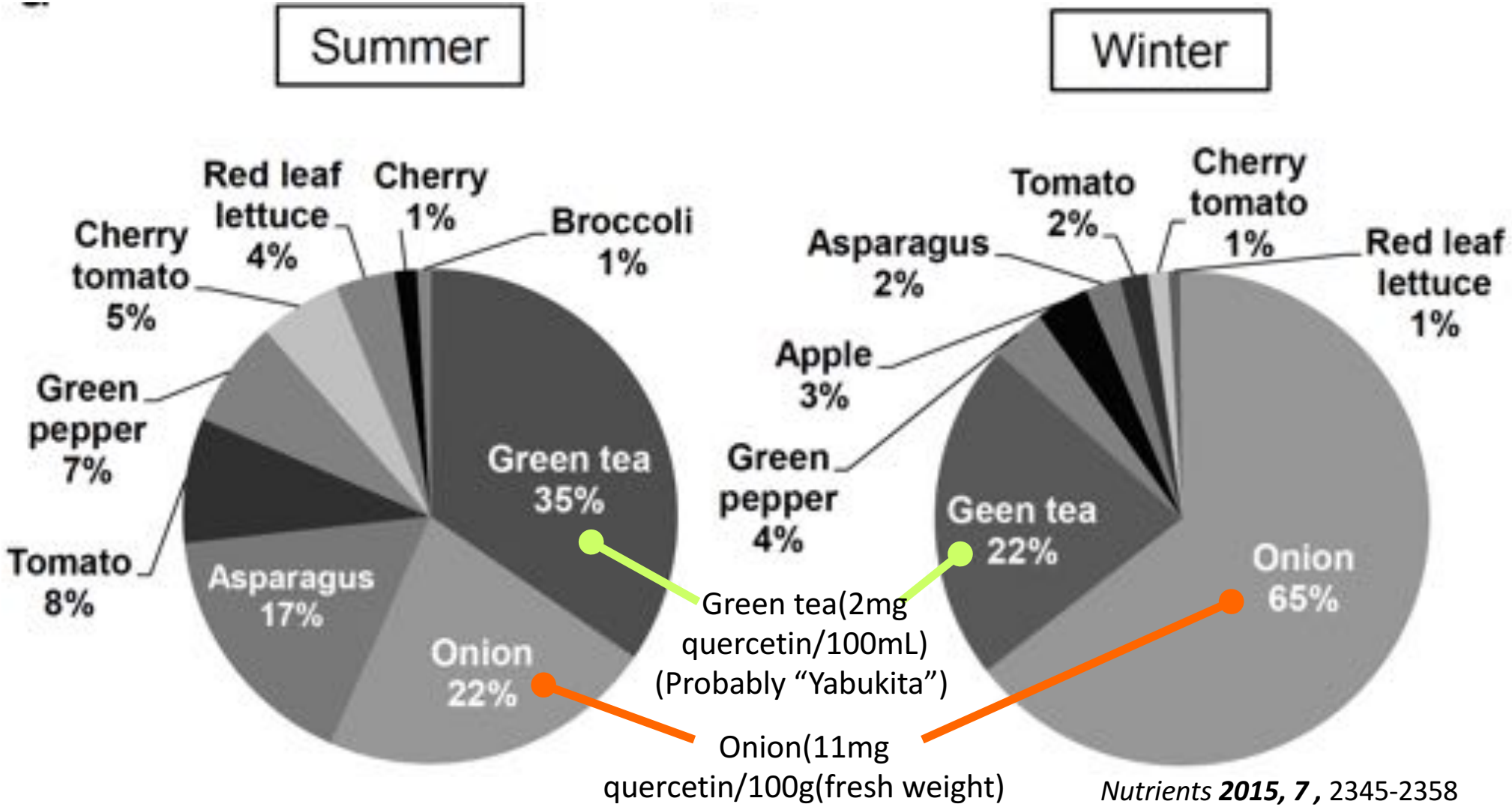
Ex. Quercetin-4'-O-β-glucoside

★ The quercetin in Japanese tea is largely quercetin-glucosyl-rhamnosyl-glucoside.



The results of diet survey in Japan (Hokkaido)

Onions and tea are important dietary sources of quercetin.



Nutrients ***2015, 7*** *,* 2345-2358

Cultivars × Quercetin

The content of flavonols is influenced by the cultivar.

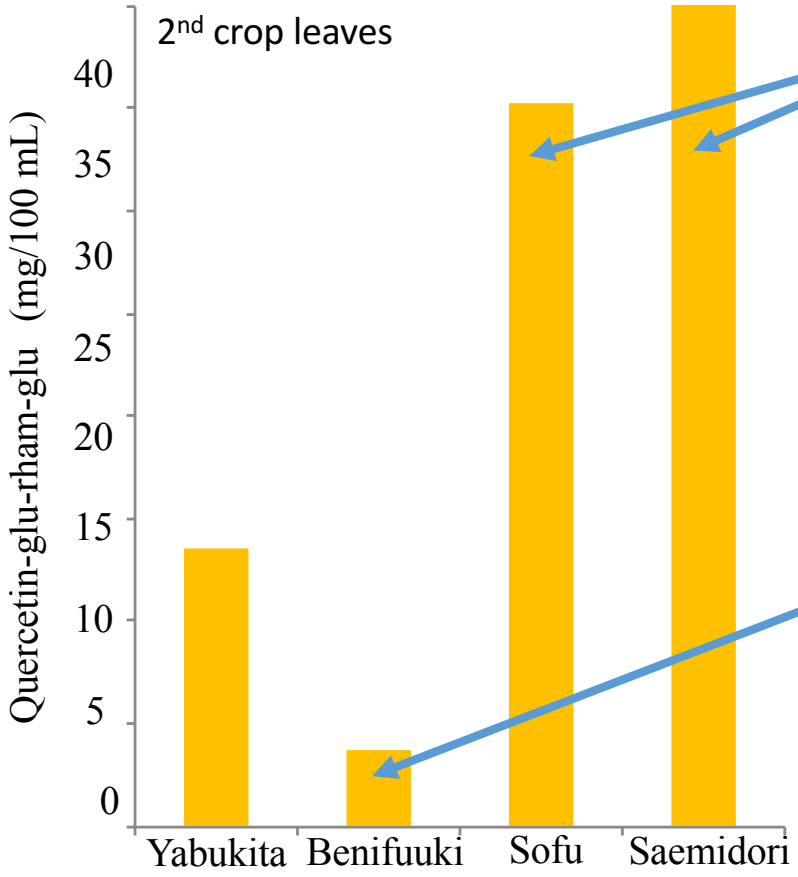


Fig. The content of quercetin-glycoside in Japanese tea cultivars.
2.5g leaves/100mL water, at 4℃ overnight.
Quercetin-3-*O*-glucosyl-rhamnosyl-glucoside
=Quercetine-glu-rham-glu

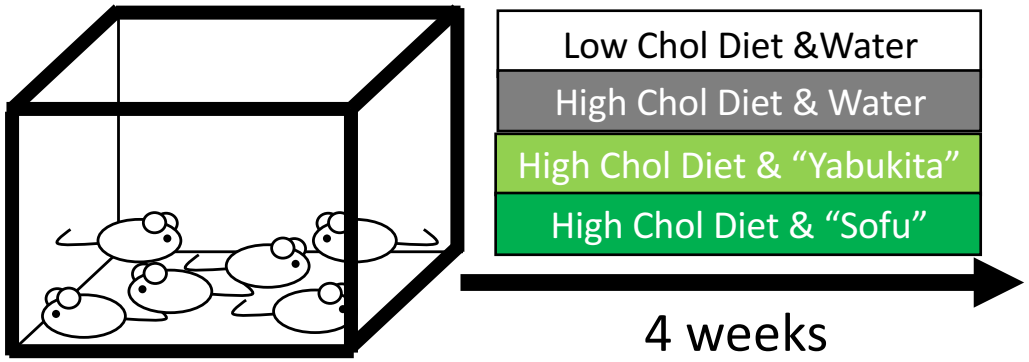
"Sofu" and "Saemidori" are typical quercetin-rich cultivars, and these cultivars contain about double quercetin content compared with "Yabukita".

"Benifuuki" is rich in catechins, but its quercetin levels are relatively low.

The quercetin-rich cultivars contribute to rise an ingestion efficiency of quercetin.

Quercetin-rich tea has a strong anti-oxidant effect.

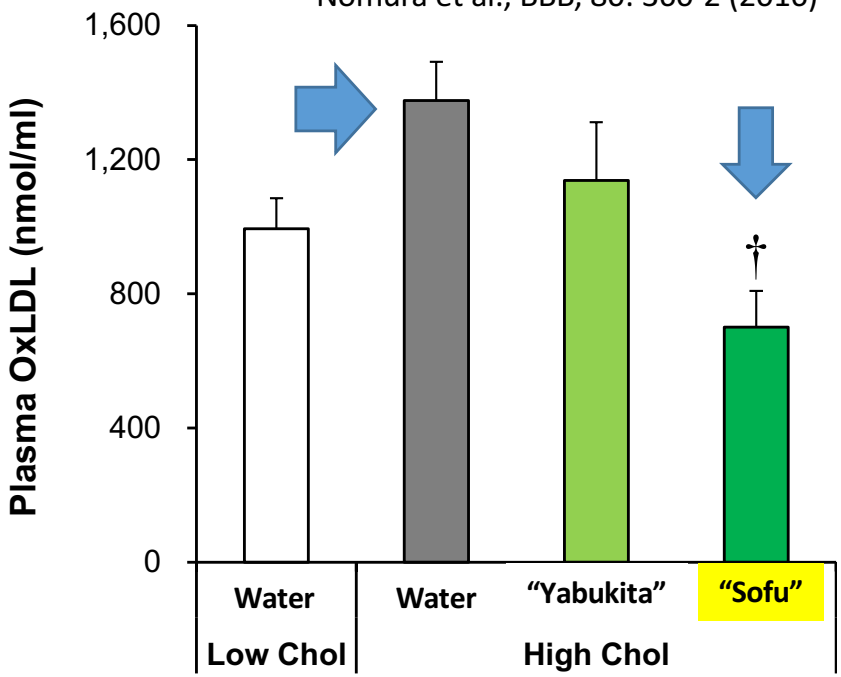
Mice were given a cholesterol diet and a test-drink for 4 weeks.



Measurement of plasma oxidised low-density lipoprotein (OxLDL) level.

OxLDL increased in High-cholesterol + water mice, but the increase in plasma OxLDL level was significantly inhibited in High-cholesterol +**"Sofu"** mice.

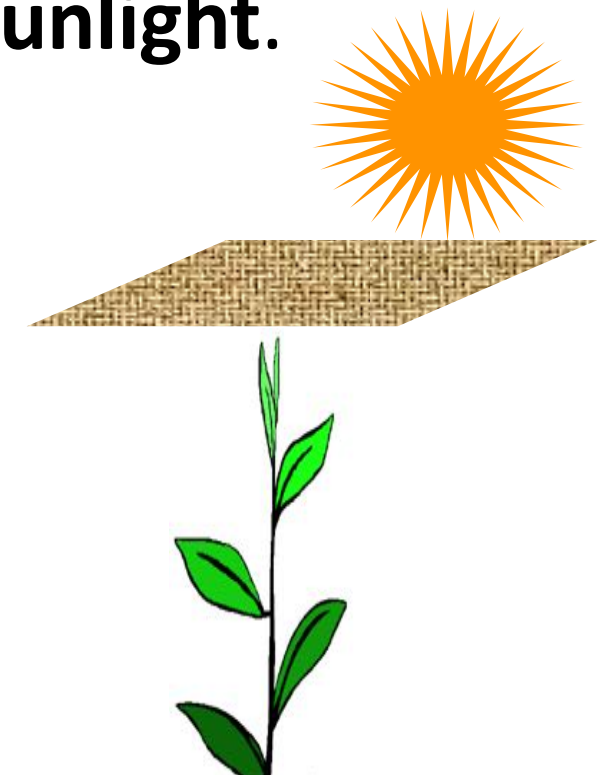
Nomura et al., BBB, 80: 360-2 (2016)



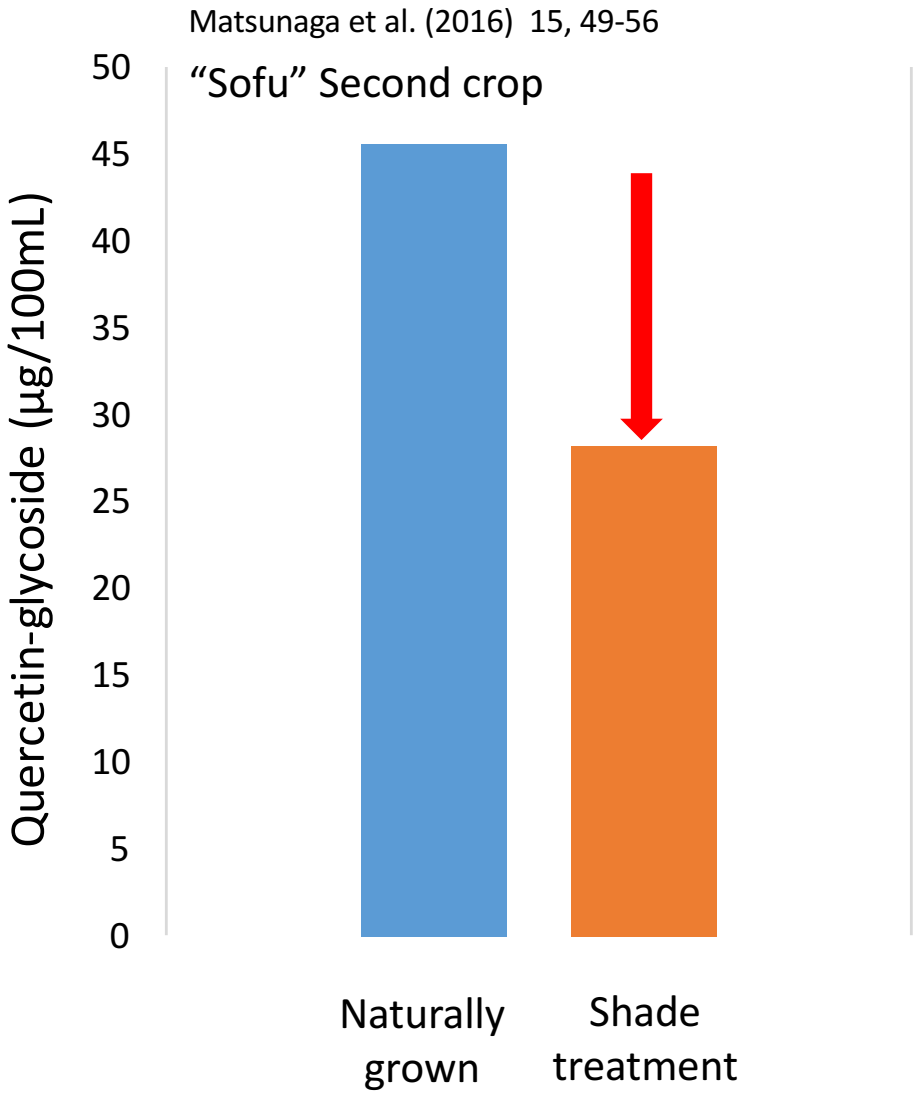
Values are mean±SEM.
†p <0.05 vs High Chol-Water (Kruskal Wallis test, n = 9-10)

Shading treatment × Quercetin

Quercetin content changes with **sunlight**.

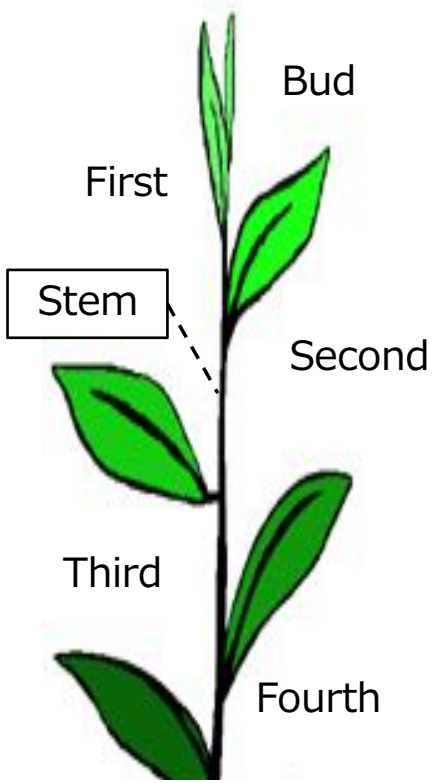


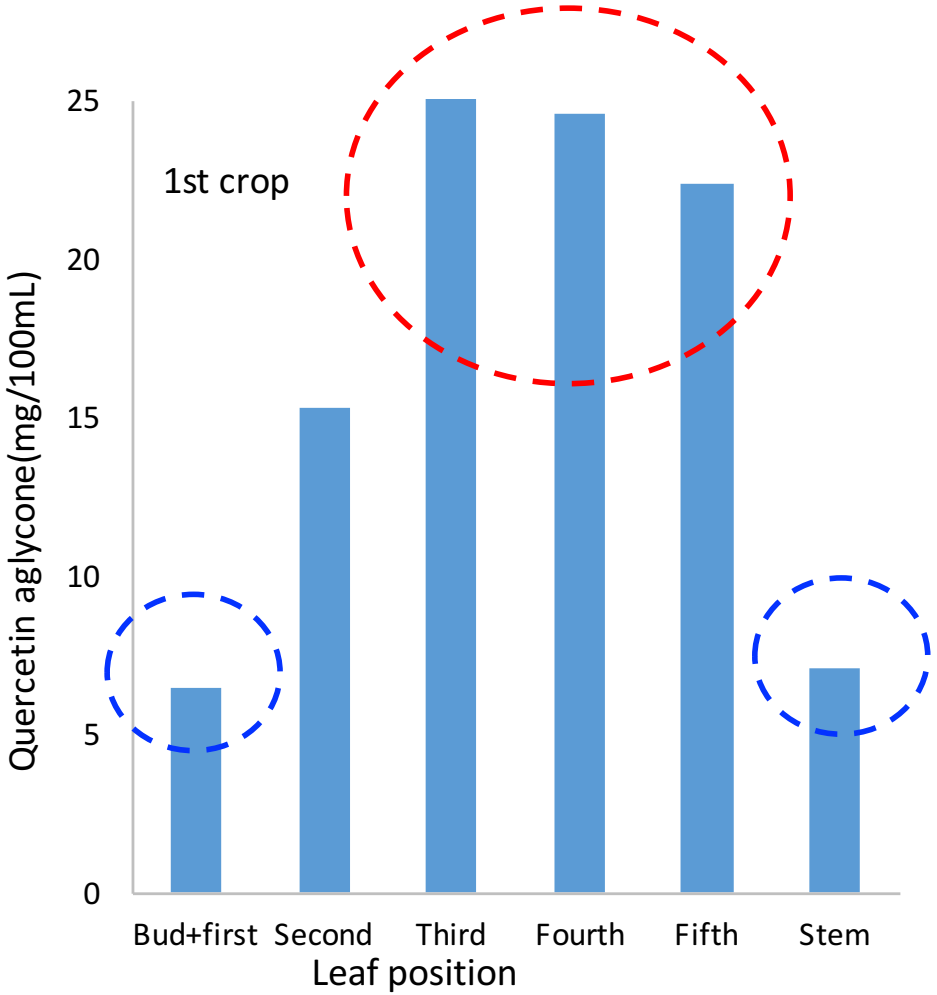
Shading treatment decreases quercetin levels in leaves.
The content of quercetin in **Gyokuro** and **Matcha** made from the shade treated leaves were comparatively low.



Maturity × Quercetin

Quercetin content changes with **leaf position**.





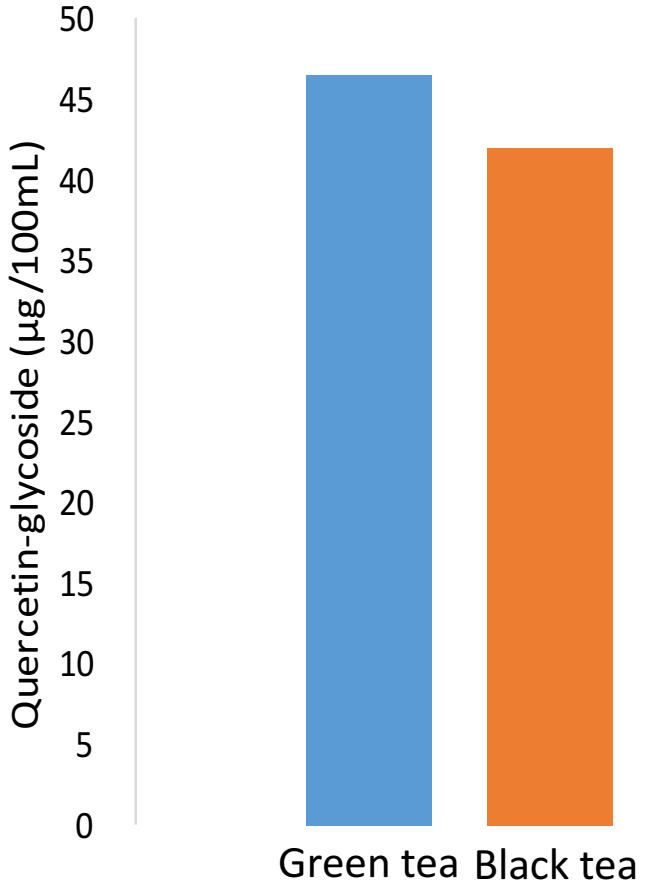
The quercetin is abundant in mature leaves.

But its levels are poor in immature leaves and stems.

Therefore, stem tea is not suitable as sources of quercetin.

Fermentation × Quercetin

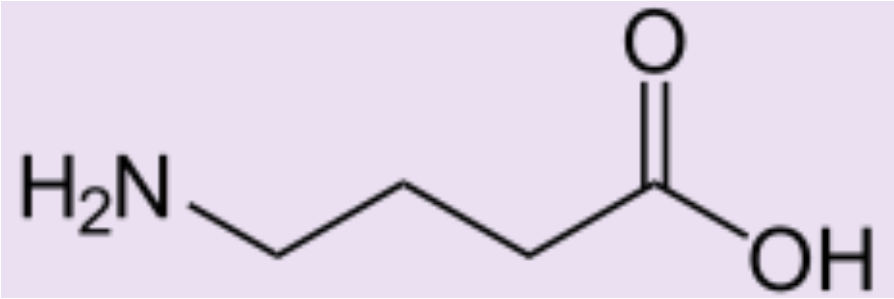
It is well-known that the fermentation process in the tea manufacturing decreases **catechin** levels.



But, the **quercetin** levels were not affected by the fermentation process.

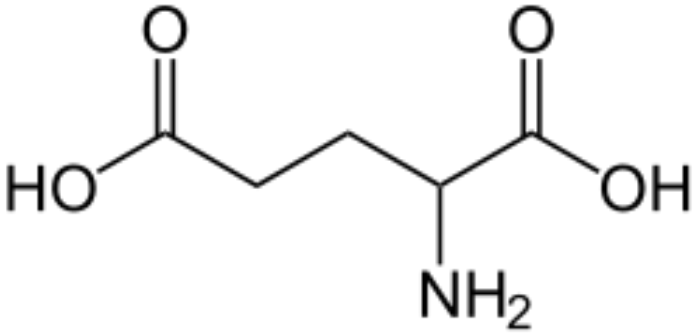
Quercetin is expected to be one of the functional ingredients in oolong tea and black tea, not only in green tea.

GABA

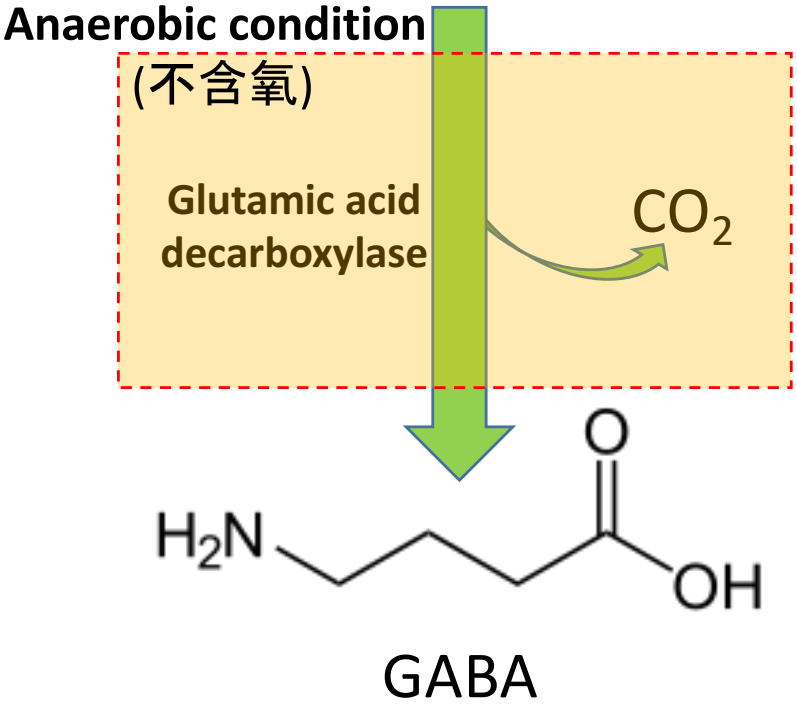


★ It has been reported that GABA induced a decrease in blood pressure.

Anaerobic treatment × GABA



Glutamic acid
(the precursor of GABA)



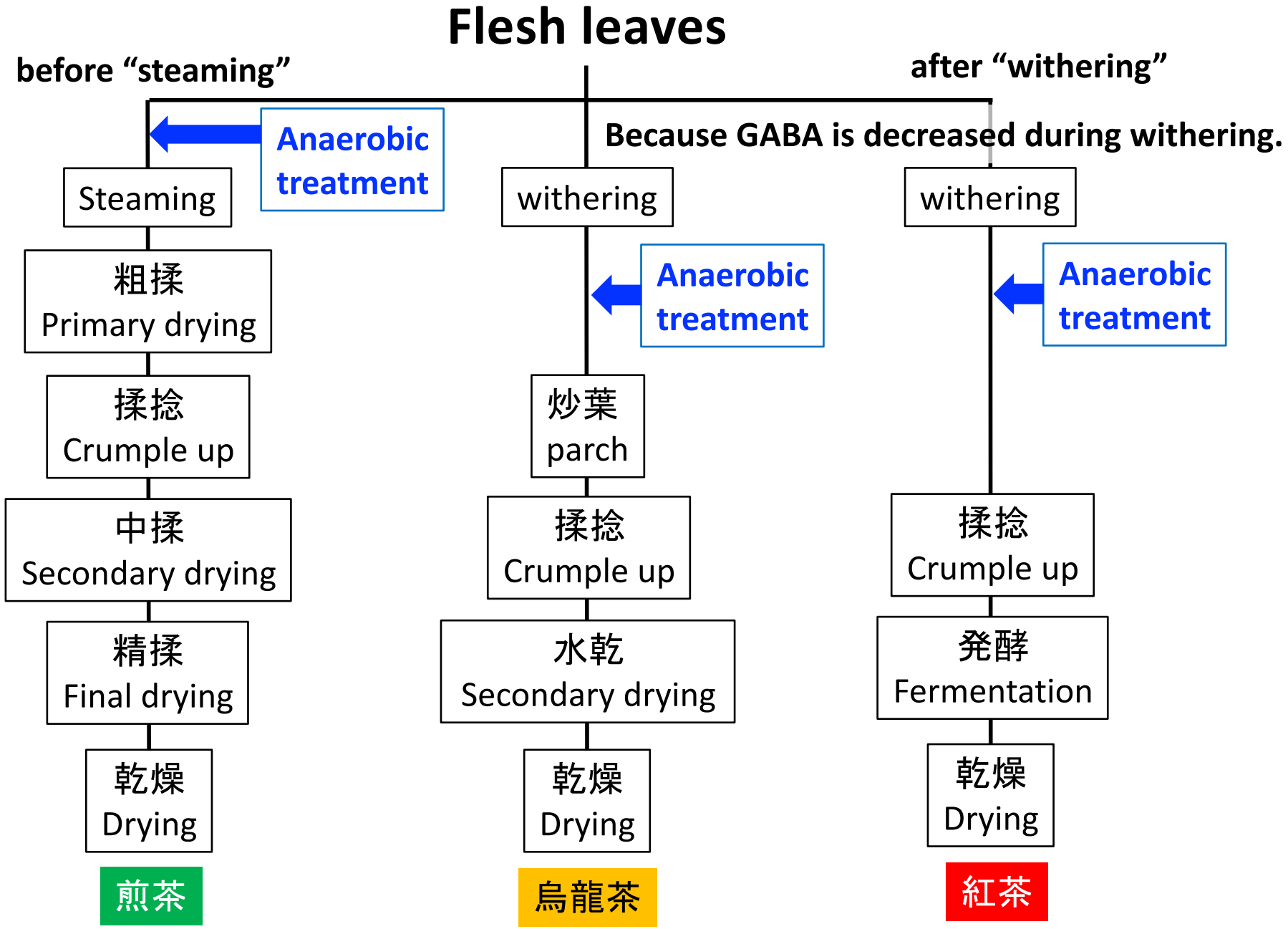
GABA

★ γ-aminobutyric acid (GABA) is not present in fresh tea leaves.

★ But, GABA is formed from glutamic acid by enzyme (glutamic acid decarboxylase) when the leaves are processed in **anaerobic condition** (incubation in nitrogen gas).

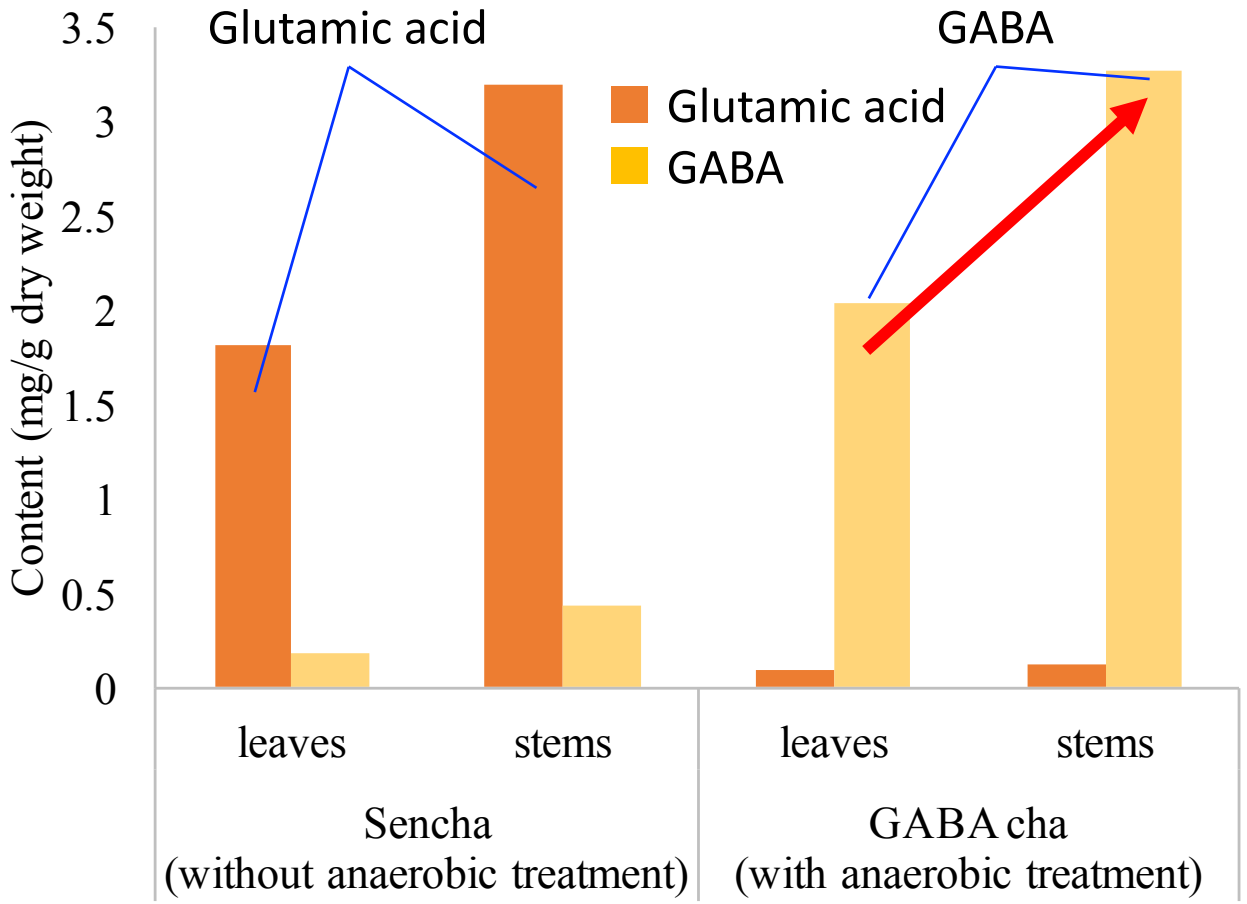
★GABA enriched-tea is called “GABA-cha” or “GABAlong-cha” in Japan.

When is the timing of anaerobic-treatment ?



Stems are important for GABAcha.

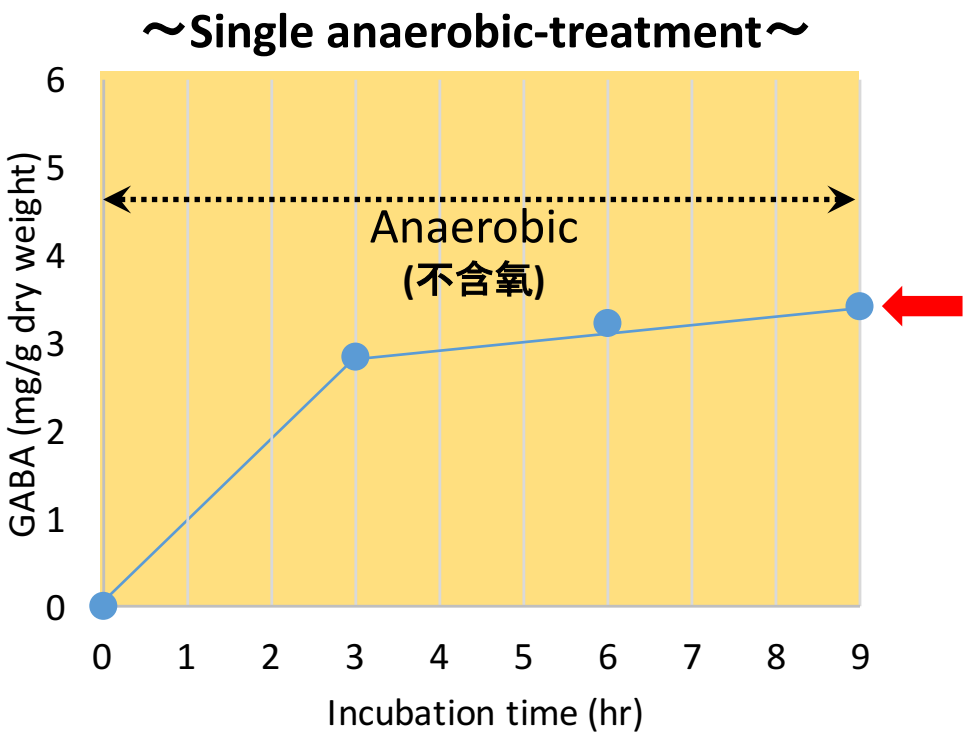
日本食品科学工学会誌 46, 274-277 (1999)

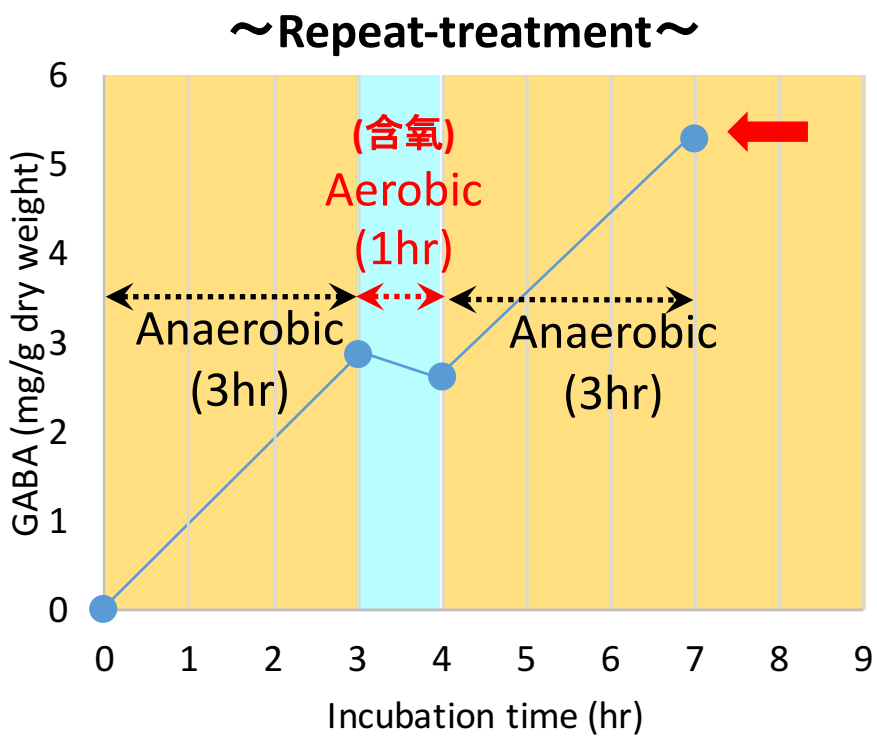


The stems contained higher glutamic acid than the leaves.

Consequently, the GABA-cha from the stems contains higher amounts of GABA than the leaves.

Anaerobic-treatment method





The repeat treatment increases the amount of GABA in the leaves compared with a single anaerobic treatment.

Agric.Biol.Chem. 51, 2865-2871 (1987)

Repeat of anaerobic- and aerobic-treatment is important.

Glutamic acid increases during aerobic-treatment.
The 3hr-1hr-3hr cycle is better.
Glutamic acid is supplied from protein while an aerobic-treatment.

Sumary ①

★ If you want to take **caffeine** and **EGCG**.

→ you should use **boiled** or **hot water**.

→ We recommend **Sencha**, **Gyokuro** and **Matcha**.

★ If you want to get the immune enhancing effect by **EGC**,

→ you should use **cold water**.

★ If you don’t want to take **caffeine**,

→ you should use **cold** or **iced water**.

→ and we recommend **Bancha**, **Hojicah** and **Stem tea**.

Sumary ②

★ If you want to get the **psychosocial stress-reducing effect** by **theanine**,

→ you should use **cold or iced water**.

→ and we recommend **Gyokuro**, the first crop **Sencha** and **Stem tea**.

★ If you want to take **quercetin**,

→ we recommend **Sencha**.
"**Sofu**" and "**Saemidori**" are better cultivars.

→ **Stem tea** and **Bud tea** are not suitable for intake of the quercetin.

★ If you want to take **GABA**,

→ you should choose the **anaerobic-treatment tea, GABAcha**.

Thank you for your attention.

