# Variations of glycosidic tea aroma precursors, volatile, β-D-glucosidase activity and respiration intensity during green tea withering.

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**Summary**

During 12-hour-term tea leaves withering, endo- β -D-glucosidase activity increased obviously, and the trend matched well with the variation of the precursors amount in tea leaves. With the extend of withering time, water in leaves evaporated gradually, and the respiration intensity decreased quickly. The amount of volatile in tealeaves grown obviously by withering 4-8 hours, then kept in a lower level. It suggested that there was a dynamic equilibrium of precursors in leaves, i.e. the increasing of volatile from precursors and the evaporating of volatile into atmosphere, which caused by a dynamic variation of biosynthesis and enzymatic hydrolysis of glycosidic aroma precursors in tealeaves during green tea withering. Glycosidic aroma precursors were not the main substrate of metabolism in tealeaves. On the contrary, it was stockpiled in withering leaves.

**Keywords**

Glycosidic aroma precursors, Volatile, β -D-glucosidase, Respiration intensity, Green tea withering

## Introduction

The aroma components of green tea are largely produced as a result of biochemical reactions induced via injury to the tea leaf (*Camellia sinensis*) structure. [1] Fresh tealeaf is virtually odourless, however, volatile aroma components are released if enzymes and substrates, separated within the cellular structure, are allowed to mix when the leaf structure is damaged. [2] The aroma of green tea contains fresh and fruity or flowery notes, monoteterpene alcohols provide a flowery or fruity contribution to tea aroma. [2]

The term "aroma precursor" generally refers to a conjugated compound capable of generating a volatile species upon hydrolysis, as opposed to the biosynthetic origins of aroma compounds. These conjugates are usually glycosidic derivatives, and by far the commonest sugar residue reported are primeveroside(6-O-β-D-xylopyranosyl- β -D-glucopyranoside) and glucoside, [3] both of the two glycosides possessing a glucopyranoside and β -linkage to the aglycone. Glycosides of geraniol, linalool, linalool oxides( I ,II), phenylethanol ,benzyl alcohol ,methyl salicylate and cis-3-hexenol have been isolated and identified from tea leaves. [4-7] The aglycones will released from their respective glycosides, by enzymatic processes, following plucking, tissue damage, or malady infection etc. When the glycosides with monoterpene alcohol and aryl alcohol as the aglycones are hydrolyzed, flowery and fruit note will generated, which are extremely important to the green tea aroma.

In recent years, there has been considerable activity with researches into the isolation and identification of tea aroma precursors, purification of glycosidase, activities and characters of glycosidase, as well as the variation of glucosidase activity and content of precursors in different tea cultivars and different parts of tea leaves. [8-13] However, there is no data available with respect to the variation of glycosidic aroma precursors during tea especially green tea manufacture. The relations among glycosidic aroma precursors, volatile, β-D-Glucosidase activity and respiration intensity during green tea withering are also ambiguous.

It was widely believed that a longer term of withering stage was necessary to an optimal pan dried green tea aroma. Early assumptions, that the amount of volatile in leaves could be improved by a longer term withering. However there was no evidence to support this proposition, since endo- enzymes activities were still active in withering leaf.

## Materials and Methods

**Tea Samples.** Fresh tea leaves of cv. Zhuye(*Camellia sinensis*) were plucked in the tea garden of Anhui

Agricultural University in May and June 1999.

**Withering Condition.** Fresh tea leaves are withered at 25℃ with the humidity of 67-72%, the withering leaves were sampled once every 2 or 4 hours.

**Volatile Samples.** Withering leaves are sampled and deactivated enzyme with microwave for 2min, dried and crushed. Dried tea powder (25g) was refluxed with boiling water (500ml). By using of Likens-Nickerson Simultaneous Distillation Extraction (SDE) equipment with ether (25ml) as solvent, 0.5ml 100ppm of ethyl decanoate was added to the tea infusion as internal standard, and the samples were kept boiling for 60min. The ether solution was treated as the volatile samples.

**Measure of Aroma Precursors.** Withering samples 20.0g cooled with liquid nitrogen and extracted with 120ml boiling alcohol overnight, 20ml 20mM citrate buffer (Ph6.0) was added into the extraction and concentrated in vacuum to less than 20ml(to remove alcohol), the same volume of ether was used to wash the extraction (to remove volatile and other impurity) for 2 times. 3g PVP was added into aqueous phase to absorb tea polyphenols, the extraction then was mixed with suitable volume of crude enzyme(tea acetone powder) and incubated at 37℃ for 2 hours in a sealed tube,15ml of ether covering on the mixture, the librated aglycone was then extract with ether for 2 times and treated to gas chromatograph.

**Gas Chromatograph.** Shimadzu GC-9A, flame ionization detector, PEG-20M column (50m × 0.25mm i.d.)_; Carrier gas: N2 (1ml/min); Column temperature: 50℃ (5min) $\xrightarrow{3℃/min}$ 190℃ (10min) ; Split ratio: 18/1.

**Monitoring of the Respiration Intensity.** Respiration intensity was measured by GXH-305 Infra –red ray respirometer at 25℃.

**Investigation of Glucosidase Activities.** β -D-glucosidase activity was measured with method we previously reported[]. 3.0g fresh withering leaves,3g PVP and suitable volume of 20 mM citrate buffer(Ph6.0) were crushed on ice, centrifuged at 10,000rpm for 5min and 13,500rpm for 15min at 4℃. The supernatant was adjusted to 15ml with buffer. 200ul of crude enzyme solution, 600ul buffer and 200ul 10mM p-nitrophenyl glucoside(pNPG) were incubated at 37℃ for 30min, then 2.5ml 1.0M NaCO3 was added into it to stop the reaction. The β -D-glucosidase activity of the samples was expressed as the OD values at 405nm.

## Results and discussion

Fresh tea leaves were withered at 25℃ with the relative humidity of 67-72%, the withering leaves were sampled once every 2 hours, Variation of water content (Table 1), aroma precursors, volatile, β-glucosidase activity and respiration intensity were investigated at the same time.

**Table 1. Variation of the water content in tea leaves during withering**

*Unite: g*

| *Treatment* | *0* | *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|---|---|
| Withering at 25℃ | 20.059 | 19.464 | 19.068 | 18.620 | 18.31 | 18.01 | 17.69 |
| Treatment | 7 | 8 | 9 | 10 | 11 | 12 | |
| Withering at 25℃ | 17.31 | 17.00 | 16.71 | 16.42 | 16.21 | 15.71 | |

**Table 2. Variation of total content of the glycosidic aroma precursors during withering**

| Withering Stages (hrs) | 1st Exp. | 2nd Exp. | 3rd Exp. | 4th Exp. | Average | Standard deviation |
|---|---|---|---|---|---|---|
| Fresh tea leaves | 100 | 100 | 100 | 100 | 100 | 0 |
| 2 | 167.6 | 120.4 | 112.2 | 87.4 | 121.9 | 33.54 |
| 4 | 107.8 | 100.2 | 108.8 | 97.74 | 103.6 | 5.56 |
| 6 | 90.3 | 144.5 | 116.4 | 97.55 | 112.2 | 24.19 |
| 8 | 113.6 | 81.9 | 97.1 | 119.2 | 102.9 | 16.88 |
| 10 | 93.0 | 79.6 | 102.0 | 128.5 | 100.8 | 20.65 |
| 12 | 127.3 | 82.3 | 96.7 | 102.5 | 102.2 | 12.44 |
| Total | 799.6 | 708.9 | 733.2 | 732.9 | 743.6 | 113.26 |

By four times experiments, it seemed that the results of aroma precursors could not be reproduced with the same method. Since we had examined the method again and again. So the deviations may come from

the different of the material itself. From the table we deduced that the content of precursors was higher in withering leaves than that in fresh tea leaves especially withered for 2 and 6 hours. (Table 2) The investigation of volatile components showed the highest content at 4 hours withering leaves. (Table 3) It would be interesting and important for green tea manufacture that four-hour-withering was necessary and optimal to obtain a pleasant green tea aroma.

**Table 3. Variation of content of volatile compounds during withering**

*Unit: 100ppm/25g dried tea*

| Withering time (hrs) | 0 | 4 | 8 | 12 | Total |
|---|---|---|---|---|---|
| Exp. 1 | 0.6178 | 1.6451 | 1.0803 | 1.0227 | |
| Exp. 2 | 0.9600 | 1.3129 | 0.9867 | 1.0834 | |
| Average | 0.7889 | 1.4790 | 1.0335 | 1.0531 | 4.3545 |
| Standard deviation | 0.2421 | 0.2349 | 0.0663 | 0.0424 | 0.5857 |

β -D-glucosidase activity during withering was investigated in a series of experiments. (Table 4) The β -D-glucosidase activity of withering leaves were higher than that in fresh tea leaves during 14-hour-term withering. But the change extend was faint and there was a rise and fall every 4 hours. The 4 experiments reproduced well.

**Table 4. Variation of β-D-glucosidase Activity during withering**

*Unit: %*

| Withering Time (hrs) | 1st Exp. | 2nd Exp | 3rd Exp. | 4th Exp. | Average | Standard deviation |
|---|---|---|---|---|---|---|
| Fresh tea leaves | 100 | 100 | 100 | 100 | 100 | 0 |
| 2 | 116.3 | 125.2 | 122.9 | 123.0 | 121.8 | 3.85 |
| 4 | 107.9 | 113.2 | 105.6 | 108.5 | 108.8 | 3.18 |
| 6 | 114.9 | 123.0 | 112.4 | 114.1 | 116.1 | 4.71 |
| 8 | 106.1 | 114.7 | 107.9 | 110.6 | 109.8 | 3.74 |
| 10 | 124.9 | 128.9 | 104.6 | 111.4 | 117.4 | 11.37 |
| 12 | 91.6 | 96.6 | 97.6 | 101.7 | 96.9 | 4.15 |
| 14 | 116.4 | 118.6 | 133.0 | 130.4 | 119.6 | 9.95 |
| Total | 878.1 | 920.2 | 884.0 | 899.7 | 890.4 | 40.95 |

As a kind of substrate sources of tea leaves respiration, the relations between the glycosidic aroma precursors and respiration intensity were still ambiguous. A gradual decline of the respiration intensity was recorded during withering. The decreasing extent at the early 2 hours was prominent, and there were still a faint increasing at 6 hours and 10 hours. (Table 5) The results of investigations confirmed that the change tendency of tea leaves respiration intensity was correspondent with its water content , and no evidence showed that there were some relations between respiration intensity and β -D-glucosidase activities.

**Table 5. Variation of the respiration intensity of tea leaves during withering**

*Unit: mg $CO_2 \cdot g^{-1} hr^{-1}$*

| Withering time (hrs) | 0 | 2 | 4 | 6 | 8 | 10 | 12 | Total |
|---|---|---|---|---|---|---|---|---|
| Exp.1 | 26.82 | 20.03 | 16.84 | 17.83 | 17.04 | 18.09 | 17.67 | |
| Exp.2 | 26.60 | 20.38 | 19.12 | 20.25 | 20.19 | 20.12 | 14.47 | |
| Exp.3 | 26.92 | 21.83 | 19.76 | 21.01 | 19.61 | 20.03 | / | |
| Average | 26.78 | 20.75 | 18.57 | 19.70 | 18.95 | 19.41 | 16.07 | 140.23 |
| Standard deviation | 0.173 | 0.954 | 1.535 | 1.661 | 1.676 | 1.147 | 1.6 | 8.746 |

During 12-hour-term withering ,endo- β -D-glucosidase activity increase faintly ,and the trend matches well with the variation of the tea aroma precursors amount in tea leaves.

With the extend of withering time, water in leaves evaporates gradually, and the respiration intensity decreases quickly. The amount of volatile in tea leaves grows obviously by withering 4-8 hours, then keeps in a lower level .It suggests there is a dynamic equilibrium of precursors in leaves, i.e. the increasing of volatile from precursors and the evaporating of volatile into atmosphere. Early assumptions, that the amount of volatile in leaves could be improved by a longer term withering, is difficult to support, in fact, a rather misleading proposition.

It can be deduced that there is a dynamic variation of biosynthesis and enzymatic hydrolysis of glycosidic aroma precursors in tea leaves during withering, Glycosidic aroma precursors are not the main substrate of respiration metabolism .On the contrary, it will be stockpiled in leaves with the increase of aglycones and free saccharides. During green tea process, the amount of glycosidic aroma precursors keeps in a high level, and remains stable in the final green tea product. (Fig 1)

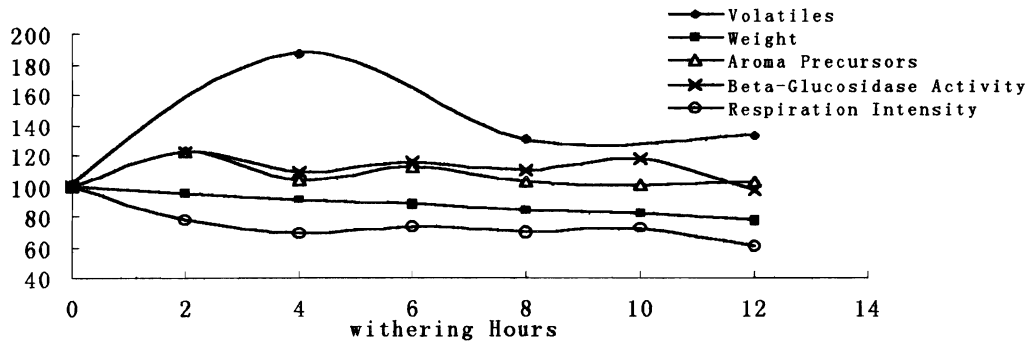


**Fig. 1 Variations of water content, aroma precursors, volatile, β-glucosidase activity and respiration intensity during withering process.**


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