Studies on aroma formation mechanisms during tea manufacturing suggest methods not only to improve the quality of made teas, but also to produce new types of teas

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Summary

Tea aroma is one of the most important factors to determine the quality of made teas. Our studies on floral aroma formation during oolong tea manufacturing including the famous Formosa oolong tea, “Oriental Beauty”, have revealed that tea plants respond to the stresses applied on them to produce many compounds to protect themselves against the stresses. One of them is tea aroma. This fact suggests possibilities to produce new attractive tea aroma by applying any type of stresses on tea leaves during tea manufacturing processes, especially during which tea leaves are alive.

Introduction

Tea aroma is one of the most important factors to determine the quality of made tea. Many studies have been done to analyze volatile constituents of tea, but a few have been carried out to show how each of the important aromas is generated during the manufacturing. We have shown how some of floral tea aromas are generated by the action of endogenous enzymes in leaves of tea plants during tea manufacturing (Sakata *et al*., 2005; Sakata 2008), suggesting the possibilities to manufacture new types of teas.

Aroma formation in fermented tea is based on self-defense systems in tea plants

Young shoots of a tea plant (*Camellia sinensis*) can be basically processed to any of the made teas (green tea, oolong tea or black tea) *via* different manufacturing processes (Figure 1). In green tea production juvenile leaves are steamed or pan-fired to kill endogenous enzymes just after being plucked. In so-called "fermented tea (oolong tea and black tea)" production, however, several kinds of treatments such as solar withering, turning-over *etc*. are carried out to let endogenous enzymes work before heating for drying, resulting in various kinds of tea aroma formation as well as coloring of tealeaves.

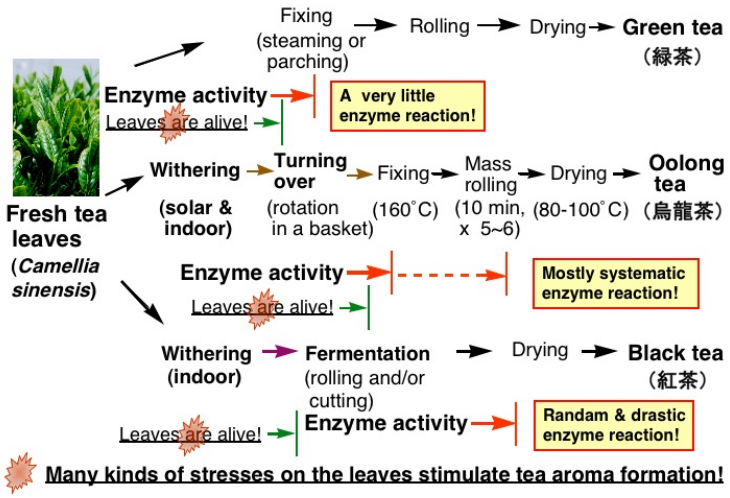

Figure 1. Outline of basic tea manufacturing.

Our studies (Sakata *et al*., 2005; Sakata 2008) have shown that benzyl alcohol, 2-phenylethanol, monoterpene alcohols such as linalool, geraniol *etc*. and so on, are stored as β-primeverosides (disaccharide glycosides: aroma precursors) in leaves of tea plants (the more in the younger leaves) and liberated from the aroma precursors during the tea manufacturing by the action of β-primeverosidase.

We found that β-primeverosidase was localized in cell wall and cavity area among cells (Figure 2). Aroma precursors (β-primeverosides) are present in vacuoles. They never encounter each other in ordinary conditions, but stresses such as insect feeding, infection by microbes and wounding let them

react to release bioactive volatiles such as geraniol, linalool, benzyl alcohol, *etc.,* that are reported to show fungicidal activities against the pathogenic fungus *Colletotricum camelliae* of tea plants. These compounds are representative tea aromas themselves. So the aroma formation in tea leaves during manufacturing oolong tea is concluded to be the results of defense responses of tea plants against various stresses.

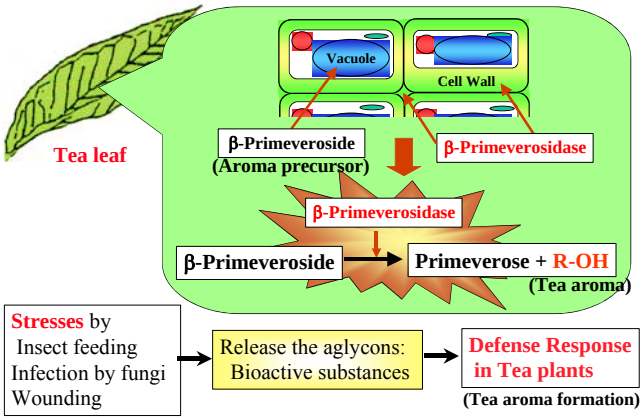

Figure 2. Volatiles emission from tea leaves as a kind of self-defense.

The reason why Oriental Beauty and Darjeeling second flash have so nice aroma

While we were studying on tea aroma formation of oolong tea, we knew that the very specific Formosa oolong tea, Oriental Beauty, is rich of aroma and its flavor is similar to that of Darjeeling second flash. Interestingly material fresh tea leaves for Oriental Beauty production must be infested by the insect, green leafhopper *Jacobiasca formosana* (Kinoshita *et al*., 2006). We were so interested in the tea aroma formation of this tea and launched a collaborative study with a team of Tea Research and Extension Station of Taiwan headed by Dr. Mu-Lien Lin.

We have prepared two series of plucked tea leaf samples, healthy and insect-infested ones, at each step of the tea manufacturing. Each sample was dried and subjected to GC-MS analysis. Surprisingly, a key compound, 2,6-dimethylocta-3,7-diene-2,6-diol (DOD), was found in the infested tea leaves as a sole prominent peak, but not in the healthy leaf sample (Ogura et al., 2008). DOD is known to be converted to hotrienol, which is responsible for the Muscatel flavor of Darjeeling second flash, by heating for drying. Further interestingly the amount of main tea aromas such as linalool, geraniol, benzyl alcohol, etc. from the infested sample were also generated very much more than those from the healthy sample as the tea processing proceeded.

We also investigated the gene expression profiles as well as the chemical profiles in tea leaves during the tea manufacturing and confirmed that the traditional method of the oolong tea manufacturing includes unique processes, that utilizes plant defense responses to elevate the production of volatile compounds and other metabolites (Cho *et al*., 2007).

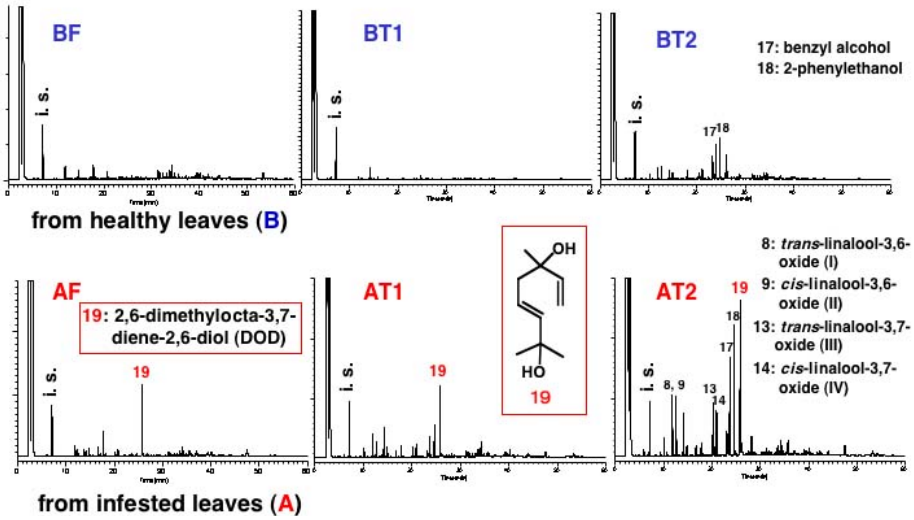

Figure 3. Comparison of volatiles liberated during the processing between healthy (B) and infested (A) tea leaves.
F, fresh leaves; T1, after the 1st turning over; T2, after the 2nd turning over

We also carried out a collaborative project with a team of Tea Experimental Station of TRA, India, headed by Dr. Mridul Hazarika and confirmed that the infestation by green flies as well as thrips were

concerned with the Muscatel flavor formation of Darjeeling second flash (Gohain *et al*., 2007).

Differences of aroma formation between oolong tea and black tea manufacturing

In the case of black tea manufacturing (cf. Figure 1), withered tea leaves are very severely clashed by the rolling processes. At this stage tea leaves are not alive anymore, but enzymes are alive. So **random enzymatic reactions** including severe oxidations occur to produce so many amounts of linalool oxides (Sakata *et al*., 2008). On the other hand, tea leaves are alive for much longer time under serious stresses such as water deficiency, injuries, *etc.* until they are heated for fixing during the oolong tea manufacturing. Here **enzymes work systematically** to produce a lot of floral aroma. This is the very important point to be pointed out. The technology to produce oolong tea rich of floral aroma is based on the stress-responsive biochemical reactions of juvenile leaves of tea plants.

Utilization of our research results:

1) Black tea production from stale green tea

Green teas become costless because of bad flavor, if they have not been stored in good conditions. We have clarified that aroma precursors (β-primeverosides of floral aroma such as linalool, *etc*.) are stored in tea leaves and still remain in the tealeaves of the stale green tea. Because in green tea manufacturing endogenous enzymes are killed by steaming or pan firing just after plucking. The stale green tea was successfully converted to a black tea by being fermented with the homogenized fresh tea leaves after properly wetted (Guo *et al*., 1992). The quality of the remade tea (black tea) was shown to be comparable with that of Keemun black tea by GC analysis as well as the sensory test

2) Manufacturing new type of green tea

In recent Japanese green tea (Sen-cha) production "freshness" has been appreciated so much that the plucked leaves are stored in a room to keep them as fresh as possible before steaming at modern factories, resulting in a green tea that smells only green note. People complain that the recent Japanese green tea does not smell so attractive as that in old days. This means that slight withering between plucking and steaming that often occurred in a conventional tea factory, gives some floral aroma that contribute to the profound nice aroma of the green tea (Sen-cha). The modern systems for Japanese green tea production must be changed to make high quality Sen-cha.

Recently a tea farmer, Mr. Yoshiaki Hiruma has succeeded in manufacturing a new type of green tea named as "Kaori Bijin" in Japanese (http://hiruma-en.ddo.jp/). The tea looks like Sen-cha, but it smells nice floral aroma. He made the tea by irradiating UV light during the withering process. UV irradiation is a big stress for the plucked tea leaves. Giving the stress on the plucked tea leaves results in the nice floral aroma formation.

3) Improving the flavor of black tea and manufacturing new types of fermented tea

Made teas can be classified based on the degree of utilizing endogenous enzymes during each tea manufacturing (Figure 4). Theoretically we could produce uncountable numbers of different types of teas between green tea and black tea by changing the degree of the endogenous enzyme utilization. We have already shown

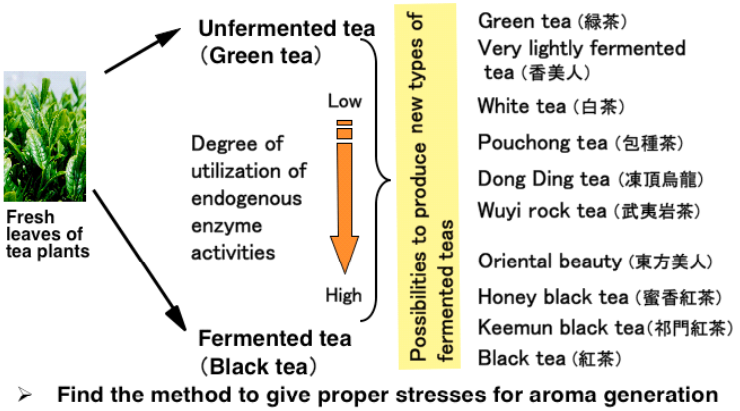

Figure 4. Possibilities to manufacture new types of tea.

that any stresses applied on the plucked tea leaves stimulate their self-defense systems to liberate volatiles, most of which are bioactive compounds. Very luckily the most of these volatiles contribute to the nice tea aroma. Mithofer *et al*. (2005) reported that continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. If we choose any desirable methods to give stresses (specific light irradiation, mechanical wounding, withering, *etc*.) on the tea leaves while the leaves are alive, we could produce a new type of tea with characteristic aroma and flavors or to improve the quality of conventional black tea.

4) Understanding the reason why tea plants grown without pesticides and fertilizers give better aroma and flavour

Plants cannot move from the place where they have germinated. To overcome the handicap, plants produce very much more secondary metabolites (some of them become chemical weapons) to protect themselves than animals do. The more the tea plants have stresses, the more they produce the secondary metabolites.

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