Tannase: A tool for instantaneous tea

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Tannase also referred to as tannin acyl hydrolase (EC 3.1.1.20), is mainly used for the bioconversion of tannic acid (hydrolysable) to nine parts of gallic acid and one molecule of sugar moiety (Lekha and Lonsane, 1997) (Fig 1). Tannase is widely present in many tannin-rich plant materials, such as myrobalan (Terminalia chebula) fruits, divi divi (Caesalpinia coriaria) pods, dhawa (Anogeissus latifolia) leaves and the bark of konnam (Cassia fistula), babul (Acacia arabica) and avarum (Cassia auriculata) trees (Lekha and Lonsane, 1997).

Microorganisms are the preferential source for production of industrial enzymes because of their biochemical process diversity and their biotechnical and economic advantages. Microorganisms can be cultivated in large quantities in a short time period by established standard fermentation process. Microbial enzymes are more stable than analogous proteins obtained from plant and animal sources. Microbes can also be subjected to genetic manipulation more readily than plants and animals. Tannase or tannin acyl hydrolase are extensively used in the food industry to produce instant tea without formation of haze, to manufacture gallic acid and propylgallate, which can be utilized as food preservatives and antioxidant, and are commonly used to remove undesirable tannins present in the manufacture of cold drinks (Chae et al., 1983).
A practice requires production of instant tea extracts which forms little or no haze when stored at refrigeration (below 4°C) temperatures. The tea extract is preferably a concentrate which is subsequently diluted to provide a ready-to-drink beverage. Haze which develops on cold storage of the extract or of tea beverages prepared from the extract is significantly reduced (Niehaus and Gross, 1997) by tannase.
The natural (healthy) tea powder, in extra-micro particle under 200–300 mesh was finished from fresh green tea, Oolong, Black tea, flower tea leaves. Normally daily consumption of tea has been coupled with reduced risk of abundant forms of cancer and other human health benefits according to some epidemiological research. Instant tea extract/powder with microbial enzyme activity will help the anti-oxidation of cells; therefore it is most beneficial in the process of aging slowing down.

Tea leaves contain many constituents, and the biological effects of tea are often attributed to the polyphenols among the tea constituents. In freshly harvested tea leaves, the following flavanols known collectively as the catechins, are present:

(+)-catechins,
(+)-gallocatechin,
(-)-epicatechins,
(-)-epicatechins-3-gallate, and
(-)-epigallocatechin-3-gallate (EGCG).

The main ingredient of green tea extracts has been shown to inhibit growth in a number of tumor cell lines such as human leukemia cell lines, mouse NFS60 cell line, MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma, UACC-375 melanoma, the prostate cancer cell lines LNCaP, PC-3 and DU145, leukemia blast cells from AML patients, and human epidermoid carcinoma cell line A431. Now a days EGCG can be obtained by isolation from green tea extracts and the yield depends on the processing and the source of the tea. Currently EGCG monomer can be a good source of anticancer agent. EGCG can be hydrolyzed by tannase enzyme and gives the final reaction product as ellagic acid with sugar molecule. The catechin in green tea act as a best natural anti-oxidation solvent, especially for the symptom of decay suffered by modern people. It shows the action to dissolve body fat and helps to provide individuals wants to lose weight an opportunity. In addition to preventing skin cancer, Green tea
helps to combat UV injury. However, keep in mind that ice tea may help crunch thirst but hot tea is more effective in preventing sunburn and skin cancer.

This instant tea powder should be stored in cool dry area and also keep away from strong direct light, maintain the temperature low. The instant green tea powder (Table 1) is applicable for manufacture of ice green tea, honey green tea and pure tea beverage of low sugar. General use to measure include at 0.15%~0.28%. The oolong tea powder is specially being applicable to manufacture the Oolong tea beverage, etc. The general use to measure the oolong tea extract is at 0.15%~0.20%. The black tea extract (Table 1) can be mixed in various foods especially to manufacture the ice black tea, milk tea, etc. General use to measure black tea includes 0.10%~0.18%.

Table 1: Instant powder extracts specifications

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Instant green tea powder/extract</td>
</tr>
<tr>
<td>Appearance</td>
<td>Yellow Powder</td>
</tr>
<tr>
<td>Soup Color</td>
<td>Yellow and Green</td>
</tr>
<tr>
<td>Total tea polyphenols</td>
<td>&gt;35%</td>
</tr>
<tr>
<td>Caffeine</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Lost after drying</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Arsenic</td>
<td>&lt;2PPM</td>
</tr>
<tr>
<td>Heavy metal(counted by lead)</td>
<td>&lt;5PPM</td>
</tr>
</tbody>
</table>
Preparation of instant tea powder

Black tea is usually prepared by subjecting freshly picked tea leaves to a series of processing conditions including the withering and rolling of freshly harvested leaves, followed by a fermentation step (enzymatic oxidation) during which much of the characteristic color, flavor and aroma of black tea are developed. The fermentation is halted after a suitable period of time by "firing" or drying the tea at temperatures ranging from about 65°C to about 100°C to inactivate the enzymes causing the fermentation. This completes the development of the flavor and color of the tea product. The extent of fermentation varies, in commercial practice, from black to various gradations between green and black. Partially fermented tea is known as "oolong" tea. Green teas are made by firing green tea before fermentation has taken place. Green, oolong, and black tea each provide a beverage having distinctive flavor and color characteristics (Table 1).

Microbial enzyme activity on instantaneous tea preparation

Enzymatic treatment of tea leaves, either continuously or batch wise, for the production of instant tea powder offers many benefits for the resulting convenience beverage product with better acid stability, color, clarity, cold water solubility, flavor and higher yield. Continuous fermentation processing of tea leaf with enzymes is an economical method of providing the necessary operating conditions of time, temperature, enzyme concentration and water for the enzymes to be effective. The high throughput needed for commercial production is also accommodated by continuous processing with minimal complexity compared to batch operation. A process for preparing black tea from green tea comprises the steps of contacting green tea with tannase in the presence of water and within a temperature range in which the tannase is active and thereafter converting the green tea to black tea in the presence of natural tea leaf enzymes and reduce the amount of tea cream produced in the conversion of green tea to black tea.
Tea is world’s most popular beverage, next to water. Every day, 800 million cups or glasses of tea are consumed globally. Hot tea is in reddish-brown colour, clear beverage, but chilling causes the formation of a haze referred to as 'tea cream' (Niehaus and Gross, 1997). The formation of this haze is generally composed of caffeine and tea flavonoids, mainly epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, and condensation products of the flavonoids formed during fermentation of the theaflavins and thearubigins. When iced tea is made at home, the haziness is either unnoticed or considered acceptable. However, when customers like to purchase iced tea: as instant powdered tea, convenience teas (instant tea with added sweetener and/or flavouring) or ready-to-drink teas (either powdered tea or tea extract mixed with water, sweetener and flavouring, and sold in cans or bottles), they prefer a product without haze (Powell et al., 1993). Tannase is an acylhydrolase with a primary catalytic activity of removing gallic acid moieties from tannins. It was originally used to produce commercial quantities of gallic acid from tannic acid, and also it is used to remove gallic acid from the polyphenols in tea, resulting in beverages that are cold-water-soluble (Niehaus and Gross, 1997).

The treatment of tea with tannase enhances the natural levels of epicatechin and gallic acid, which in turn favours the formation of epitheaflavic acid, which is responsible for the bright reddish colour of tea. This means that the treatment of tea products with tannase yields tea with a good cold-water solubility and colour. The reaction that follows is a deesterification between galloyl groups and various compounds in unconverted tealeaves (Fig 2). When the pH of the tannase enzyme mixed with other enzymes is risky to obtain the higher tannase activity to high yield of gallic acid. It is believed that the gallic acid content of the instant tea product is in part indicative of the degree of solubility, with higher levels of gallic acid correlating to better solubility. Tannase results in increased gallic acid or gallic acid salt levels from modification of polyphenolic type biopolymers such as theaflavins and thearubigins.
Tannase has been used along with laccase for the treatment of grape juice and grape musts to remove phenolic substances for chemical stabilization of the beverage. Moreover, 50% of the color of the wine is due to the presence of tannins; however, if these compounds are oxidized to quinones by contact with the air, they could form an undesirable turbidity, which causes severe quality problems. The use of tannase has been proposed as the best solution to this problem (Powell et al., 1993).

![Figure 2. Transesterification of tannic acid to propylgallate in the presence of n-propanol](image)

**CONCLUSION**

In summary, there is clearly much scope for the industrially important enzyme tannase for the removal of undesirable waste materials (tannins) present in the beverages and food materials and has the application of medical research such products anti-oxidants, anti-bacterial drug. Tannase has been shown to be a very versatile enzyme and finds application in the food, beverage, industrial and pharmaceutical industry, however due to insufficient knowledge about the enzyme, the difficulties in establishing cost-effective scaling up and downstream processing protocols the large-scale application of tannase is currently still limited. It will be exciting to witness the future developments in basic research as well as biotechnological applications of this versatile microbial enzyme.
REFERENCES


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