Effect of Beta-glucosidase on the Aroma of Milky Tea Beverage

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Abstract. The bottled milky tea in the market has been extracted and sterilized at high temperature resulting in poor aroma, favour deteriorated and other issues. In order to solve these problems, we added β-glucosidase(β-G) after high temperature sterilization and bottle-filling of milky tea beverage. It is proved to be scientific and efficiency of β-G in improving the aroma of milky tea beverage with black-tea, then the effect is the best when the concentration of β-G was 7.4 U/mL, and the amount of substrate was 100 mL, with 50 ℃ temperature and pH 5.6, as well as the reaction time is 2 hours. Sure, the innovation of this research is to obtain the effect principle and technology of enhancing aroma of milky tea through the treatment of exogenous β-G, which fundamentally solves the problem of serious loss of aroma air flow of milky tea, and ultimately realizes the maintenance of tea aroma after milky tea list and maximizes the maintenance of original tea flavour. Perhaps due to the improvement of milk tea quality, it can promote the sales of tea beverages.

1. Introduction

Nowadays, there are a variety of canned liquid milky tea beverage on the market that are popular with consumers for the affordable price and convenient. The aroma of milky tea beverage is an important indicator to evaluate its quality, which is one of the key and difficult points in the quality study, as well as a criterion for consumers in pursuit of high-quality tea beverages to judge beverage. However, during the production of milky tea beverage, especially after two processes, i.e. tea extraction and high temperature sterilization, these lead to aroma substances lose severely, and the canned milky tea beverage is far less delicious than the freshly prepared milky tea beverage. ([2, 7]) Therefore, how to improve the aroma of milky tea beverages is a pressing technical problem that needs to be solved in beverage industry.

In the study of tea aroma, we know there are not only various volatile aroma substances, but also a considerable amount of bound aroma precursor substances in bound form in the tea -- glycoside substances, for example. These fragrance-free substances will generate volatile natural aroma substances as they are hydrolyzed when contacting with acid or enzyme. In the initial stage of tea processing, the glycosidase hydrolyzes glycoside compounds in the fresh leaves, dissociates flavonoid and terpene glycosides and produces the substances that lead to the formation of aroma, taste and color of the tea.
soup ([8]). Most importantly, the content of bound aroma substances in form of glycoside in the tea leaf is much higher than that of the free aroma substances ([6],).

β-glucosidase (β-G) can hydrolyze non-reducing β-D-glucose bonded at terminal, and release β-D-glucose and the corresponding aglycone, which serves as the main enzyme to catalyze and hydrolyze glycosides and terpenes that can release aroma in tea leaves ([1]). There have been many studies about the effects of β-G on the aroma in tea leaf. As early as in 1980, researchers used crude enzyme which was extracted from purified fresh tea leaves to obtain β-D-galactosidase and β-D-glucosidase, and confirmed the existence of which glycosidase promotes the production of aroma for the first time ([5]). Later, foreign researchers cultivated homogenate from fresh tea leaves at 40 °C for 30 days, and then found a large amount of new aroma substances such as linalool and geraniol generated ([3]).

β-G, can be used as an exogenous additive to change the type and proportion of the aroma substances in tea soup, thereby improving the aroma quality and promoting the formation of fragrance. Studies have shown that the content of glycoside in black-tea is about 43% lower than that in fresh tea. This is partly because the glycoside in tea leaf is hydrolyzed by endogenous β-glucosidase and also implies that most of the glycoside is not hydrolyzed completely ([4]).

Although β-G enhances the fragrance of tea leaf and tea soup, it is still unknown whether it is effective for milky tea beverages. Therefore, in this experiment, we added β-G to the sterilized bottled milky tea beverage, then extracted and analyzed the components by headspace solid-phase micro-extraction combined with gas chromatography-mass spectrometry, to investigate the effects of exogenous β-G on the aroma of milky tea beverage. Expect to provide a scientific basis for improving the aroma of canned milky tea beverages.

2. Materials and Methods

2.1 Test Materials and Instrument

Milky tea beverage (Formula is from preliminary experiment and the black-tea is summer tea originated in Qingyuan, Guangdong, China), Citric Acid (Food-Grade, 100%, Jogel), β-G (7.4 U/mg, β-G from almonds), NaCl (0.32 g/mL)

Electronic Scales PL303, Desk-type Thermostatic Oscillator THZ-C, Thermostatic Magnetic Stirring Apparatus 85-2, Deionized Water Purifier Spring-S15, Thermostat Water Bath HWS28, Rotational Viscometer BROOKFIELD, Instrument for examination and evaluation of tea leaf(including tea tasting cup, tea tasting bowl, casserole), transparent glass beverage bottle, glass stirring rod, 25mL volumetric flask, 1mL liquid gun, pH meter, Gas Chromatography-Mass Spectrometer (Agilent 7890-5975), Extraction Handle (Supelco).

2.2 Pre-experiment and Treatment

Pre-experiment: We did a preliminary experiment to find an optimum pH value. It was that added different quality of citric acid into 100mL milky tea beverage, and measured the pH of milky tea beverage (test three times and take the average), and evaluate and review (15 professional reviewers).

In the results, we know that the pH of milky tea beverage without citric acid is 6.8. When 0.08 g citric acid was added into the milky tea beverage, the pH decreases to 5.4, and there was flocculation and precipitation. The overall taste of milky tea beverage is becoming thinner gradually with the addition of citric acid. But the color of milky tea beverage turns yellow and bright. Accordingly, considering that suitable pH for β-G and stability of milky tea beverage (As flocculation may occur during the process.), we added 0.07 g citric acid to every 100mL milky tea beverage, and then the pH went to 5.6.

Treatment: Prepare 1 mg/mL β-G solution accurately (use it right after it is ready). And add solution into the bottled pH-adjusted milky tea beverage respectively, as well as put it at 50 °C thermostat for 2-hour oscillation. After the enzyme reaction, insert the extraction needle into the bottled milky tea beverage under natural conditions to extract the aroma for 1 hour. The different treatments of samples as follow (table 1):
Table 1: Treatments on different samples

| Sample | Treatments                                      |
|--------|------------------------------------------------|
| 1(CK)  | -                                              |
| 2      | Sterilized, no pH-adjusted, without enzyme     |
| 3      | Sterilized, the optimum pH, without enzyme     |
| 4      | Sterilized, the optimum pH, with 0.1 mL β-G    |
| 5      | Sterilized, the optimum pH, with 0.3 mL β-G    |
| 6      | Sterilized, the optimum pH, with 0.6 mL β-G    |
| 7      | Sterilized, the optimum pH, with 1.0 mL β-G    |

2.3 Extraction and Measurement of Aroma

Extraction of Aroma: HS-SPME/GC-MS. Put the sample into 100 mL extraction bottle and seal it. Insert CAR/DVB/PDMS extraction head that has been activated for 5 mins at 250 °C into the sample bottle, headspace volume is 30 mL, and extract for 60 min at 50 °C under the condition of magnetic stirring.

GC Condition for adding saturated NaCl (0.32 g/mL); N6890 (Agilent); Chromatographic Column: HP-5(30 m×320 μm×0.25 μm nominal) elastic quartz capillary vessel column; Injection temperature: 250 °C, FID detector temperature: 280 °C; Carrier Gas: high-purity Nitrogen (99.999%); Flow Velocity: 1.0 mL/min; Hydrogen 30 mL/min; Air 400 mL/min; Auxiliary gas 25 mL/min. Keep column temperature at 40 °C for 2 min, and increase the column temperature to 110 °C at the speed of 2 °C/min and keep column temperature at 110 °C for 2 min, then increase the column temperature to 170 °C at the speed of 3 °C/min and keep the column temperature at 170 °C for 2 min, increase the column temperature to 220 °C at the speed of 4 °C/min and keep the column temperature at 220 °C for 2 min, and last, increase the column temperature to 260 °C at the speed of 10 °C/min and keep the column temperature at 260 °C for 5 min; SPME extraction head is thermally resolved at GC injection for 6 min.

MS Condition: Chromatographic Column: DB-5MS (30 m×0.25 mmID×0.25 μm film thickness); Carrier gas: Helium; Injection Temperature: 250 °C; Pause Splitless Injection 1μl column flow velocity: 1 ml/min. Interface temperature of chromatography-mass spectrometry: 280 °C; Ion source temperature: 230 °C; Quadrupole rod temperature: 150 °C; Ionization: EI; Electron energy: 70 eV.

Quantitative Method of Aroma Substances: The relative amount was adopted. The relative amount of each compound isolated was calculated based on the ratio of the peak area of each compound to the total peak area.

2.4 Method of Sensory Evaluation

15 professional reviewers review and evaluate the milky tea beverage. It was found that the treatment had a great influence on the color and taste in the practical operation process, so evaluation is according to three aspects: color of soup (20%), aroma (40%) and taste (40%), similar as the evaluation of tea soup. The full grade is 100 points.

3. Results and Analysis

3.1 The aroma measurement of milky tea beverage with different concentration of enzyme

GC-MS online search (all of chromatograms on the appendices) and materials were adopted to analyze the aroma substance component from sample 1(S1) to sample 7(S7), citric-acid-treated milky tea beverage, and reacted 15 min at 50°C, then extracted the aroma under natural condition, to explore the optimal additive amount of β-G based on online results searched and analyzed. After the aroma compounds in each sample were obtained, we analyzed variety numbers and percentage of different fragrance categories. The results are as follows:
As fragrance similar to lavender. Of course, the seven samples also have common aroma like flowers; 2-

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Table 2. Constituent and proportion of milky tea beverage aroma with different treatments

| categories | S1 Proportion (%) | S2 Proportion (%) | S3 Proportion (%) | S4 Proportion (%) | S5 Proportion (%) | S6 Proportion (%) | S7 Proportion (%) |
|------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| alkane     | 2.35             | 6.39             | 5.48             | 6.78             | 9.96             | 7.59             | 4.02             |
| alkyne     | 0.59             | 0.80             | 0.94             | 2.66             | 2.30             | 2.56             | 4.31             |
| alcohol    | 13.15            | 12.65            | 9.21             | 11.19            | 12.73            | 13.77            | 22.59            |
| aldehyde   | 7.99             | 3.30             | 3.85             | 10.60            | 12.32            | 13.97            | 8.83             |
| ketone     | 10.44            | 9.60             | 5.80             | 6.37             | 6.35             | 9.63             | 8.87             |
| ester      | 1.22             | 2.28             | 1.11             | 0.74             | 0.55             | 0.81             | 1.43             |
| ether      | 0.01             | 0.00             | 0.39             | 0.07             | 0.00             | 0.00             | 0.04             |
| total      | 34.75            | 34.55            | 26.78            | 39.21            | 43.94            | 45.48            | 38.09            |

After GC-MS online search, we found 73 kinds of aroma substances in Sample without β-G, and 85 kinds of aroma substances milky tea beverage with adding β-G of different amounts (Sample 4, 5, 6, 7), which means there are 12 new aroma substances generated. Also, the total number of aroma substances in each sample with β-G is higher than samples without β-G, and each milky tea beverage has its own unique aroma substances different from other groups. Such as, Linalool oxide have a strong woody flavor and fragrance of the flowers which was found in Sample 5 and 6 but failed to be found in Sample 4 and 7. Also β- cyclocitral was only found in Sample 6 which has fruity odor and floral aromas, similar to that of violet ketone, iris and wood fragrance. And terpinolene was only found in Sample 5 which has the odor of lemongrass and is always used as one of the main spices. Tetrahydrolavandulol was only found in the Sample 7, which naturally exists in lavender and some natural essential oils, and is a little spicy and has fragrance similar to lavender. Of course, the seven samples also have common aroma substances which were main aroma substances with a large proportion in the milky tea beverage. such as 3, 7-dimethyl-1, 5, 7-octatriell-3-ol (2.3%~4.4%) has sweet fragrance like flowers; 2-heptanone (4%~4.5%) has fruity odor similar to that of pear and so on.

From the table 2, With the addition of citric acid, the proportion of aroma substances in the milky tea beverage significantly decreased from 35.75% to 26.78. In addition, among the four samples with β-G, the maximum number of aroma substances detected was 45, and the minimum number was 38, while only 35 aroma substance were detected in sterilized milky tea beverage treated with citric acid without β-G. Therefore, we can see the damage of processing to the aroma of milk tea beverage.

As most effective components of aroma substances are alcohols, the aroma substances that accounted for the highest proportion in all samples were all alcohols. On the contrary, the content of ether is the lowest, even there is no ether in samples 2, 5 and 6. In addition, the proportion of alkanes and aldehydes increased first and then decreased with the increase of β-G. However, the proportion of alcohols showed a trend of increase obviously.

Combine with the GC-MS online search, the contents of cis-5-Ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol, benzaldehyde and 2-heptanone all in ranked top 4 in the samples 4, 5, 6, and 7 that shows they are the dominant aroma substances. In seven samples, the content of 2-heptanone is very high which is naturally found in cheese, strawberry, and coconut, with a fruity and sweet aroma similar to that of the cream. On the contrary, benzaldehyde took up a low proportion in the aroma substances of the milky tea beverage without β-G which naturally exists in hyacinth, citronella, cinnamon, irises and roses, with the aroma of bitter almond.

Totally, the proportion of aroma substances treated with enzyme gradually increased, up to 50.09%, twice as that of the unprocessed milky tea beverage (26.78%).

3.2 Effects of Different Enzyme Concentration on Sensory Quality of Milky Tea Beverage

15 reviewers made an evaluation by sensory evaluation method and scored according to its’ soup color (20%), aroma (40%) and taste (40%). We deducted the highest and lowest marks, and took the average of the rest of the scores as the final score. The results are as follows:

![Fig. 1 The scores of each index of different samples](image)

From figure 1, S5 and S6 got the highest aggregates scores and the aroma of the S5 was better while the taste of the S6 was better, but there was no significant difference in four samples. The taste of milk tea beverage becomes better with the increase of β-glucosidase content. From figure 1-c, the color of S5 was best, followed by S7. To sum up, samples with citric acid had better color of soup, except for the unprocessed milky tea beverage.

| Sample | Final Score | Comments on Aroma |
|--------|-------------|-------------------|
| 3      | 84.0        | Natural aroma, plain taste |
| 4      | 85.7        | Natural aroma, plain taste |
| 5      | 89.8        | Natural, mellow milk aroma, fresh and sweet |
| 6      | 90.4        | Aromatic, strong fragrance, strong fruity flavor |
| 7      | 86.9        | Aromatic, pungent |

From table 3, S5 had the natural aroma with mellow milk aroma and sweetness of tea, which was rated highest. Combined with figure 1, it can be seen that S5 and S6 all got high scores, and had the natural aroma with mellow milk aroma and sweetness of tea. But S7 got the lowest score which added the highest level of β-G. Therefore, we believed heavier aroma of milky tea beverage did not mean better beverage, the coordination and balance between various aroma substances counted.

4. Discussion and Conclusion

1. Add citric acid into milky tea beverage can keep its beautiful color of soup, but the content of major aroma substances including alcohols and aldehydes decrease significantly after adding citric acid and sterilization, which lowered the aroma quality. This problem is aroma loss in the extraction of traditional milky tea beverage.

2. The environment where exogenous β-G is active is adjusted by citric acid. In order to keep the β-G within the range of reactivity, and consider the appearance and pH of beverage, it was finally determined that the amount of citric acid was 0.07 to make a pH 5.6 of milky tea beverage.

3. β-G has the function of aroma enhancement, and β-G of different amounts added into milky tea beverage plays different in aroma enhancement. There are 12 aroma substances generated in the milky
tea beverage with β-G, and the proportion of various aroma substances is significantly changed. The content of alcohols increased with the increase of the amount of β-G, up to 22.59%. Alkanes, aldehydes, ketones and other major aroma substances also change to different content. Combined with the results of GC-MS online search and sensory evaluation, the optimal amount of β-G added is 0.6ml or 100 ml. But the cost of β-G is high, the amount of 0.3ml β-G can be considered as the best addition amount which obtained 91.0 points on fragrance score.

The comprehensive experimental results show that the β-G is useful in aroma enhancement and taste improvement and verified its scientific and efficiency in improving such technical problems as aroma loss and poor taste during the traditional production. And the effect was the best when the concentration of β-G was 7.4 U/mL, and the amount of substrate was 100 mL, with 50 ℃ temperature and pH 5.6, as well as the reaction time is 2 hours. which can solve such technical problems as aroma loss and poor taste during the traditional production of milky tea beverage.

**Appendices**

Chromatogram comparison on milky tea beverage with different treatments

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[Chromatogram images]

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