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Aroma changes of black tea prepared from methyl jasmonate treated tea plants*

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Abstract: Methyl jasmonate (MeJA) was widely applied in promoting food quality. Aroma is one of the key indicators in judging the quality of tea. This study examined the effect of exogenous MeJA treatment on tea aroma. The aroma components in black tea prepared from MeJA-treated fresh tea leaves were extracted using headspace solid-phase microextraction (HS-SPME) and were analyzed using gas chromatography-mass spectrometry (GC-MS) and GC-olfactometry (GC-O). Forty-five volatile compounds were identified. The results revealed that the MeJA-treated black tea had higher levels of terpene alcohols and hexenyl esters than the untreated tea. Moreover, several newly components, including copaene, cubenol, and indole, were induced by the MeJA treatment. The activities of polyphenol oxidase and β-glucosidase in fresh tea leaves changed after the MeJA treatment. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis indicated that the gene expression levels of polyphenol oxidase and β-primeverosidase were upregulated by two and three folds, respectively, by the MeJA treatment ($P<0.01$); however, the gene expression of β-glucosidase was downregulated to a half level. In general, the aroma quality of the MeJA-treated black tea was clearly improved.

Key words: Aroma, Black tea, Methyl jasmonate (MeJA), Headspace solid-phase microextraction (HS-SPME), Gas chromatography-mass spectrometry (GC-MS), Gas chromatography-olfactometry (GC-O), Gene expression

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1 Introduction

Tea (*Camellia sinensis*) is the most widely consumed non-alcoholic beverage in the world, with several different types available, such as green tea, oolong tea, and black tea. Black tea is a kind of fully fermented tea and is manufactured in four distinct stages, including withering, rolling, fermentation, and drying (Tomlins and Mashingaidze, 1997). The worldwide popularity of black tea has been attributed to its sensory quality, with particular emphasis on its unique aroma and characteristic flavor. Hence, the specific aroma profile is a key factor in determining the quality grade of tea.

Aroma compounds are key contributors to tea flavor perception. A variety of different volatile organic components (VOCs) are presented in tea although in minute quantities (i.e., 0.01% of the total dry weight). VOCs have a significant impact on tea flavor because of their low threshold values and resulting high odor units (Rawat *et al*., 2007). Generally, aroma compounds are classified into two groups: Group I is defined as non-terpenoid species, for instance, hexanal, *trans*-2-hexenal, 2-hexanol, and

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cis-3-hexenol; Group II is defined as terpenoid species, such as linalool, linalool oxides, benzaldehyde, phenylacetaldehyde, nerol, geraniol, α-ionone, benzyl alcohol, phenyl ethanol, and β-ionone (Muthumani *et al.*, 2013). The ratio of terpenoid species to non-terpenoid species has been used to classify black teas for their flavor quality. In particular, linalool and geraniol, which belong to the terpene alcohol family, always impart a sweet, flowery aroma on black tea (Sanderson *et al.*, 1973; Vitzthum *et al.*, 1975; Kawakami *et al.*, 1995).

It is well known that the aroma composition of tea varies with various parameters, such as plant variety, plucking season, soil, climate, pre- and post-harvest treatments (Qin *et al.*, 2013). Moreover, the flavor of prepared tea could also be affected by insect infestation, stresses, and mechanical injury to tea plants (Bailey *et al.*, 2005; Bhattacharyya *et al.*, 2007; Cho *et al.*, 2007). Methyl jasmonate (MeJA), a well-characterized fatty acid-derived cyclopentanone signal, can induce a series of changes in secondary metabolites in a wide range of plants (Alvarez *et al.*, 2009; Kuźma *et al.*, 2009; Qiu *et al.*, 2009; Chen *et al.*, 2011). MeJA plays an important role in promoting the quality of agricultural products, especially in improving the aroma qualities of certain fruits and vegetables. Kuroyanagi *et al.* (1998) isolated seven sesquiterpenoid phytoalexins from the culture medium of hairy roots of *Hyoscyamus albus* following its treatment with MeJA. Loivamäki *et al.* (2004) also reported that jasmonate induction led to an increase in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuate*. Moreover, the impacts of MeJA on the biosynthesis of volatile compounds in climacteric and non-climacteric fruits, such as apples and strawberries, respectively, have also been reported (Ayala-Zavala *et al.*, 2005; Kim *et al.*, 2006; de la Peña Moreno *et al.*, 2010a; 2010b; Belhadj *et al.*, 2008; Gohain *et al.*, 2012). However, investigations toward the effects of exogenous MeJA on tea aroma have been scarcely reported.

In this study, the aroma components of black tea prepared from MeJA-treated tea plants were extracted using headspace solid-phase microextraction (HS-SPME) and analyzed using gas chromatography-mass spectrometry (GC-MS) (Lin *et al.*, 2013). Moreover, the characteristic aroma components of the prepared tea were further identified using GC-olfactometry (GC-O) (Lv *et al.*, 2012). To determine the mechanism of action, we studied the enzyme activities and gene expressions of several aroma-related enzymes, such as polyphenol oxidase (PPO), β-glucosidase, and β-primeverosidase. After clarifying the variation of tea aroma induced by exogenous MeJA, this study could provide a theoretical basis and technical support for using an exogenous inducer to enhance aroma quality and developing a new flavored black tea.

2 Materials and methods

2.1 Reagents

MeJA (>98%) was purchased from Sigma-Aldrich Co. (USA). Pure water was obtained from a Milli-Q purification system (Millipore, USA). A 65-μm SPME head [(polydimethylsiloxane/divinylbenzene (PDMS/DVB)] fiber was obtained from Supelco (Bellefonte, USA). The RNAplant plus kit, FastQuant RT kit (with gDNase), and SuperReal PreMix Plus (SYBR Green) were purchased from TIANGEN Biotech Co., Ltd., China.

2.2 Tea samples and preparations

Longjing 43, a cultivar of the tea plant (*Camellia sinensis*), was grown in the garden of the Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China. Samples were treated and prepared in spring. All the experiments were carried out in triplicate and separately in March, April, and May.

Preparation of MeJA-treated black tea: 1000 individual tea plants were homogeneously sprayed with 4 L 0.25% (w/v) solution of MeJA dissolved in ethanol. The fresh tea leaves were then allowed to stand for 24 h before being plucked (one bud with the second and third leaves). The leaves (2000 g) were then processed as follows: withered for 45 h at room temperature, rolled for 40 min in a proper rolling machine, and then subjected to a fermentation process before being dried at 120 °C to provide the finished black tea (500 g).

Untreated black tea was used as the control. It was prepared using water instead of MeJA for spraying the tea plants, and other processings were the same as the above described MeJA-treated black tea.

2.3 HS-SPME procedure

Extraction of the volatile compounds was performed by an HS-SPME method using a PDMS/DVB fiber. Before analysis, the fiber was preconditioned for 5 min in the injector of the GC as indicated by instructions of the manufacturer.

For each sample, 10.0 g of tea, previously homogenized, was weighed in a 150-ml vial and infused with 30 ml of boiling water. The vial was then sealed with tetrafluoroethylene and immediately heated to and held at 60 °C. Prior to extraction, an incubation time of 5 min was applied to equilibrate the fiber. The SPME fiber was then successively exposed to the headspace for 60 min while maintaining the sample temperature at 60 °C. Following the sampling process, the SPME fiber was injected into the GC injector and left for 3.5 min to allow for the thermal desorption of the analytes.

Based on our trials, the best extraction conditions were defined as follows: a 65-μm SPME head (PDMS/DVB) fiber with extraction time of 60 min, an extraction temperature of 60 °C in a water bath, and a 1:3 brewing ratio of tea to water.

2.4 GC-MS analyses

GC-MS analyses were conducted on an Agilent-6890 GC directly coupled to an Agilent HP 5973 MSD ion trap mass spectrometer (Agilent, USA). An injector temperature of 250 °C was used in the splitless injection mode. An HP-5MS column (30 m× 0.25 mm i.d., 0.25 μm film thickness) was used for the analyses. The oven temperature was programmed as follows: the initial temperature was held at 50 °C for 5 min, and the column was then heated at 3 °C/min to 210 °C and subsequently held for 3 min before being heated at 15 °C/min to 230 °C and finally to 250 °C. Helium (purity >99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min.

The mass spectra were collected in the electron impact ionization mode, with an electron energy of 70 eV, interface temperature of 280 °C, ion source temperature of 230 °C, quadrupole temperature of 150 °C, mass scan range of 35–400 atomic mass units, and an emission current of 34.6 μA. Each compound was identified using the National Institute of Standards and Technology (NIST) 98 library based on their linear retention indices (LRI) and authentic standards. The relative proportions of the constituents were obtained by flame ionization detector (FID) peak area normalization. Quantitative results were obtained using the following equation: relative content (%)=single constituent area×100%/total area.

2.5 Sensory evaluation of tea aroma

Small portions (3.0 g) of the MeJA-treated and the untreated black tea were accurately weighed and placed in 150 ml of boiling water to brew the tea for a period of 5 min. Five professional tea tasters were then invited to perform a sensory evaluation and objective assessment of the aroma of tea.

2.6 Determination of activities and gene expressions of enzymes in fresh tea leaves

2.6.1 Activities of PPO and β-glucosidase

PPO and β-glucosidase were extracted and their acitivities were determined according to Wang *et al.* (2001) and Wakuta *et al.* (2010). However, because of a lack of β-primeveroside as the substrate, the activity of β-primeverosidase was not determined.

2.6.2 Gene expressions of PPO, β-glucosidase, and β-primeverosidase

Freshly prepared tea leaves (100 mg) were immediately ground in liquid nitrogen, and a portion of this sample powder was used for RNA extraction with the RNAprep Pure Plant Kit (TIANGEN Biotech Co., Ltd., Beijing, China). Complementary DNA (cDNA) was synthesized using the FastQuant RT Kit (TIANGEN Biotech Co., Ltd., Beijing, China). Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using SuperReal PreMix Plus (SYBR Green) with the first-strand cDNA as a template on the ABI 7500 Real-Time PCR System (Applied Biosystems). The relative gene expressions of PPO, β-glucosidase, and β-primeverosidase were determined.

2.7 Statistical analysis

Data were statistically analyzed by SPSS statistical package (Version 16.0 for Windows). One-way analysis of variance (ANOVA) was used to test the hypothesis and the significant differences were considered when $P<0.05$. Data were expressed as mean± standard deviation (SD).

3 Results

3.1 Identification and quantification of volatile components in MeJA-treated and untreated teas

Table 1 shows the differences in the relative content of each volatile compound extracted from the MeJA-treated and untreated black teas. A total of 42 major volatile compounds were identified using GC-MS and were grouped into different classes, including 9 terpene alcohols, 7 terpenes, 3 newly induced components, 7 hexenyl esters, and 16 other components. As shown in Fig. 1, significant differences of most volatile compounds were observed between the MeJA-treated and untreated black teas. Increases in the production of these compounds could eventually enhance the sensory quality of freshly prepared black tea.

The flavor index was calculated according to Yamanishi *et al.* (1968a; 1968b) as the ratio of the sum of relative distributions of Group II compounds to the sum of relative distributions of Group I compounds. The flavor index of the MeJA-treated black tea increased to almost 2.65; however, that of the untreated black tea was only 2.15. Based on this consideration, the sensory quality of MeJA-treated black tea was clearly improved.

3.2 Differences among terpene alcohols

Nine different terpene alcohols were found in the prepared black tea (Table 1). Geraniol was found to be the most abundant of these nine alcohols, followed by β-linalool, the levels of which were both increased due to the MeJA treatment. The total relative content of β-linalool and its oxides, together with geraniol, was 63.91% in the untreated black tea, whereas the corresponding value in the MeJA-treated black tea was 68.20%. The total content of terpene alcohols in the MeJA-treated black tea was significantly higher than that of the control ($P<0.05$; Fig. 1). It was reported that β-linalool and geraniol play important roles in the formation of black tea aroma (Schuh and Schieberle, 2006). β-Linalool and its corresponding oxides contribute vitally to the formation of the sweet aroma of black tea, whereas geraniol has an effect on the formation of the floral aroma of black tea. In this study, MeJA could induce the production of terpene alcohols, especially β-linalool and geraniol, which contributed significantly to the total percentage of volatile compounds, resulting in an improved aroma quality and possibly accounting for the sweet and floral aroma to a great degree.

3.3 Differences among hexenyl esters

Hexenyl esters are the second important class of compounds presented in the black teas (Table 1). The relative contents of these seven hexenyl esters also changed following the MeJA treatment. Notably, the relative content of *cis*-hexanoic acid 3-hexenyl ester increased from 2.6% to 3.2% due to the MeJA treatment. Furthermore, a pair of diastereoisomers, *cis*-hexanoic acid 3-hexenyl ester and 3-hexenyl-hexanoic acid ester, were presented in the overall volatile composition. The ratio of *cis*-hexanoic acid 3-hexenyl ester to 3-hexenyl-hexanoic acid ester was about 5:1 in the untreated black tea, whereas this ratio dropped to about 3:1 in the MeJA-treated black tea. Moreover, our previous research indicated that *cis*-hexanoic acid 3-hexenyl ester could be responsible for the floral aroma of freshly prepared black tea (data unpublished). Appropriate aroma and flavor components always yield higher quality of tea. In a similar research conducted on the MeJA-treated strawberries, MeJA could affect the enantiomeric distribution of ethyl 2-methylbutanoate, which also exerted a significant impact on aroma formation of strawberries (Blanch *et al.*, 2011). Likewise, it is speculated that MeJA treatment of black tea in the current study affected the enantiomeric distribution of hexanoic acid-3-hexene ester. MeJA treatment could play a significant role in affecting the aromas of different black teas, depending on different ratios of the isomers.

3.4 Differences among other volatiles

It was interesting to note that MeJA could induce several new secondary metabolite components, which was in agreement with previous studies (Rodriguez-Saona *et al.*, 2001; Martin *et al.*, 2003; Degenhardt and Lincoln, 2006). Three new components, copaene, cubenol, and indole, were induced in the MeJA-treated tea but not in the untreated tea; their relative contents were 0.21%, 0.28%, and 0.15%, respectively. However, these components were not detected by GC-MS in the untreated tea. Based on our previous GC-O results, copaene could provide a floral aroma and cubenol could provide a clean and fresh aroma, whereas indole provides a pungent aroma.

Table 1 GC-MS identification of black tea prepared from MeJA-treated and untreated tea plants

Aroma compound	LRI	Relative content (%)		*P*	Identification method
		Untreated	MeJA-treated		
Terpene alcohol					
β-Linalool	1080	13.73±0.50	15.15±0.45	<0.05	LRI, MS, Std
cis-Linalool oxide (furanoid)	1087	4.30±0.78	4.95±0.27		LRI, MS, Std
trans-Linalool oxide (furanoid)	1073	9.67±0.34	10.97±0.28	<0.05	LRI, MS, Std
cis-Linalool oxide (pyranoid)	1174	0.91±0.10	0.70±0.06	<0.05	LRI, MS tent.
trans-Linalool oxide (pyranoid)	1167	1.80±0.12	1.80±0.10		LRI, MS tent.
3-Hexen-1-ol	858	1.41±0.06	0.46±0.13	<0.01	LRI, MS, Std
Phenylethyl alcohol	1114	2.81±0.21	2.34±0.49		LRI, MS, Std
cis-Geraniol	1229	27.7±0.82	28.87±0.07		LRI, MS, Std
cis-Nerolidol	1573	1.58±0.10	2.23±0.12	<0.01	LRI, MS, Std
Hexenyl ester					
cis-Butanoic acid, 3-hexenyl ester	1212	0.46±0.08	0.53±0.09		LRI, MS, Std
cis-3-Hexenyl *iso*-valerate	1238	2.64±0.07	2.62±0.36	<0.01	LRI, MS, Std
n-Valeric acid *cis*-3-hexenyl ester	1230	0.56±0.08	0.63±0.05		LRI, MS, Std
cis-Hexanoic acid, 3-hexenyl ester	1369	2.60±0.22	3.13±0.04	<0.05	LRI, MS, Std
3-Hexenyl-hexanoic acid ester	1371	0.49±0.20	1.15±0.10	<0.05	LRI, MS, Std
trans-2-Hexenyl caproate	1391	0.31±0.10	0.28±0.14		LRI, MS, Std
cis-3-Hexenyl benzoate	1615	0.37±0.17	0.53±0.09		LRI, MS, Std
New component					
α-Copaene	1378		0.16±0.06	<0.01	LRI, MS, Std
Cubenol	1650		0.21±0.10	<0.01	LRI, MS, Std
Indole	1294		0.20±0.08	<0.01	LRI, MS, Std
Terpene					
β-Myrcene	981	2.67±0.37	2.82±0.14		LRI, MS, Std
α-Cubebene	1354	0.35±0.11	0.39±0.05		LRI, MS, Std
β-Cubebene	1388	0.36±0.13	0.37±0.05		LRI, MS, Std
α-Cedrene	1432	0.45±0.07	0.41±0.04		LRI, MS, Std
Caryophyllene oxide	1589	0.14±0.13	0.41±0.12	<0.01	LRI, MS, Std
α-Farnesene	1505	0.14±0.14	0.30±0.11	<0.01	LRI, MS, Std
δ-Cadinene	1528	0.26±0.62	0.48±0.15	<0.05	LRI, MS, Std
Other component					
Salicylic acid, methyl ester	1185	4.77±0.64	5.53±0.43		LRI, MS, Std
Benzaldehyde	960	3.30±0.13	1.63±0.26	<0.01	LRI, MS, Std
Benzene acetaldehyde	1049	3.67±0.17	2.81±0.16	<0.01	LRI, MS, Std
Decanal	1200	1.45±0.10	0.34±0.08	<0.01	LRI, MS, Std
β-Cyclocitral	1208	0.57±0.20	0.22±0.09		LRI, MS, Std
cis-Jasmone	1406	0.67±0.10	0.85±0.08		LRI, MS, Std
Geranyl acetone	1456	3.33±0.38	1.59±0.41	<0.05	LRI, MS, Std
β-Ionone	1493	1.10±0.48	0.42±0.14		LRI, MS, Std
2,6-Di-tert-butyl-4-methyl phenol	1512	0.57±0.10	0.61±0.13		LRI, MS, Std
2-Methylnaphthalene	1884	0.56±0.16	0.52±0.12		LRI, MS, Std
Tetradecane	239	1.54±0.25	1.07±0.11		LRI, MS, Std
2,6,10,14-Tetramethyl-heptadecanoic	1872	0.60±0.12	0.56±0.05		LRI, MS, Std
n-Pentadecane	253	0.75±0.10	0.65±0.06		LRI, MS, Std
1-Hexadecene	1587	0.55±0.10	0.35±0.12		LRI, MS, Std
n-Hexadecane	272	0.52±0.10	0.59±0.10		LRI, MS, Std
2,6,10,14-Tetramethyl-pentadecane	1707	0.33±0.17	0.26±0.10		LRI, MS, Std

Data are expressed as mean±SD of three replicates. LRI: linear retention index (calculated on HP-5MS column). MS tent.: tentatively identified by MS; Std: chemical standard. When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification

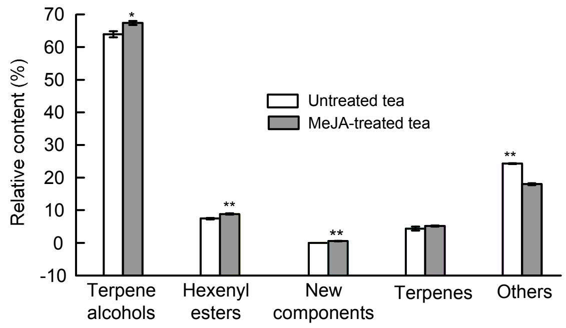

Fig. 1 Relative contents of different classifications of volatile components presented in MeJA-treated and untreated black teas
Data are expressed as mean±SD (*n*=3). * $P<0.05$; ** $P<0.01$

The terpenes in the MeJA-treated black tea, including β-myrcene, α-cubebene, β-cubebene, α-cedrene, caryophyllene oxide, α-farnesene, and δ-cadinene, also differed considerably from those of the untreated tea. The relative contents of caryophyllene oxide, α-farnesene, and δ-cadinene in the MeJA-treated black tea were significantly higher (Table 1; $P<0.05$).

As shown in Fig. 1, each of these compound classes underwent changes by the MeJA treatment, clearly indicating that MeJA had a significant impact on the formation of the overall tea aroma.

3.5 Sensory evaluations of aromas of the MeJA-treated and untreated teas

Based on the results from the five tea tasters, it became clear that the sensory quality of the aroma of black tea prepared with the MeJA-treated fresh leaves was enhanced relative to the control. As shown in Table 2, the MeJA-treated black tea was given a score of 91.7 in the sensory evaluation, whereas the untreated black tea was given a score of 88.7. Furthermore, the types of aromas were quite different: the MeJA-treated black tea possessed a honey aroma, whereas the control had a sweet aroma which was similar to normal black tea. These results therefore demonstrated that MeJA treatment induced differences in the aroma of the tea as well as enhancing the sensory quality of the aroma.

3.6 Activities of PPO and β-glucosidase

As shown in Fig. 2, MeJA treatment significantly improved the acitivty of PPO ($P<0.01$), while decreased the activity of β-glucosidase ($P<0.01$).

3.7 Gene expressions of PPO, β-glucosidase, and β-primeverosidase

PPO, β-glucosidase, and β-primeverosidase were the three most important enzymes in the release of free aroma from tea leaves. The results of qRT-PCR showed that the gene expression levels of PPO and β-primeverosidase were significantly upregulated in the MeJA-treated tea leaves ($P<0.01$). However, the gene expression of β-glucosidase was downregulated by the MeJA treatment (Fig. 3). These results were in accordance with the activities of these enzymes.

Table 2 Sensory evaluations of MeJA-treated and untreated black teas

Tea	Sensory remark	Score
Untreated	Sweet aroma	88.7±0.5
MeJA-treated	Honey-sweet aroma	91.7±1.5*

Data are expressed as mean±SD of three replicates. * $P<0.05$

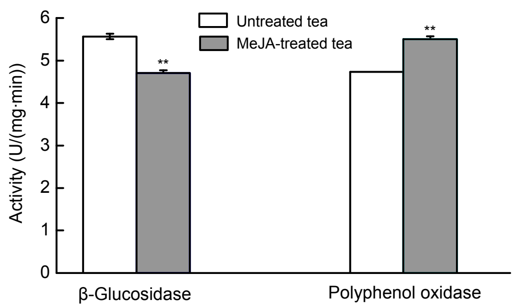

Fig. 2 Effects of exogenous methyl jasmonate on the activities of β-glucosidase and polyphenol oxidase
Data are expressed as mean±SD (*n*=3). ** $P<0.01$

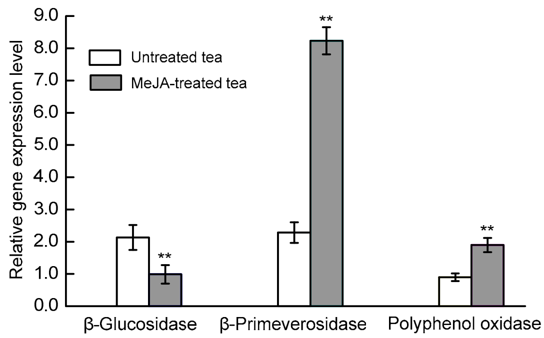

Fig. 3 Effects of exogenous MeJA on gene expressions of β-glucosidase, β-primeverosidase, and ployphenol oxidase in tea leaves
Data are expressed as mean±SD (*n*=3). ** $P<0.01$

4 Discussion

Some other studies proved the theory that tea aroma arose partly from the fresh leaves and partly from the manufacturing process. Although special manufacturing processes give black tea a unique floral, fruity, and honey-sweet aroma, to date, little attention has been given to exogenous effects on fresh tea leaves which affect the final black tea aroma. This study is the first to demonstrate that the aroma of black tea prepared from MeJA-treated tea plants is greatly improved.

The basic structure of MeJA is effectively a type of cyclopentane ketone which is a naturally volatile compound itself. MeJA could be hydrolyzed by an esterase enzyme in plant cells into jasmonate, which then works in the jasmonate pathway (Lyons *et al.*, 2013). Importantly, this pathway could then exert a significant influence on certain aroma-related enzymes, especially β-glucosidase, β-primeverosidase, and PPO (Wakuta *et al.*, 2010; Yang *et al.*, 2013).

In this work, MeJA treatment could significantly enhance the gene expressions of β-primeverosidase and PPO, although suppressed the gene expression of β-glucosidase in fresh tea leaves. β-Primeverosidase and β-glucosidase are both crucial catalysts for the hydrolysis reactions of the glycoside precursors of the corresponding free aroma compounds (Wang *et al.*, 2001; Sarry and Günata, 2004). Tea volatile components exist, to a significant degree, as glycoside-bound aromas (glycoside precursors) in fresh tea leaves. Only after coming into contact with hydrolases, mainly β-glucosidase and β-primeverosidase, can these conjugated aroma compounds be released as the free type during the hydrolysis reaction (Ogawa *et al.*, 1997; Ijima *et al.*, 1998). Among the glycosidic aroma precursors, β-primeveroside is usually considered the prevalent glucoside. Modulation of gene expression may result in improving enzyme activity. It can be deduced that the improved activity of β-primeverosidase is mainly responsible for the release of terpene alcohols, such as β-linalool, linalool oxides, and geraniol, which account for the greatest proportion of tea aroma. That is, MeJA could potentially induce the enhancement of β-primeverosidase activity, which ultimately results in releasing terpene alcohol aromas. As for PPO, the mechanism seems much more complicated. PPO could oxidize catechins, the predominant chemical components in tea, into the corresponding oxidized catechin species. These oxidized catechins could then undergo condensation reactions with other types of catechins to generate theaflavins (TF) or even thearubigins (TR). Furthermore, the oxidized catechins could affect β-carotene, resulting in its degradation to β-ionone, which has a woody and floral aroma. In addition, the oxidized catechins could react with other carotene species to form ketone aroma compounds (e.g., β-ionone or α-ionone). The compounds produced from carotenoids also have a remarkable effect on the aroma of tea (Ravichandran, 2002). Hence, we deduced that the MeJA treatment on fresh tea leaves has a positive effect on several crucial aroma-related enzymes that ultimately result in the promotion of black tea aroma quality.

In the present study, MeJA has been used as an exogenous inducer to improve the gene expressions and the activities of PPO and β-primeverosidase in tea leaves. The activity promotions of these enzymes eventually had an effect on the sensory quality of the tea aroma. The characteristic aromas of the black tea prepared from MeJA-treated fresh tea leaves were identified using an HS-SPME method combined with GC-MS. The results provide good qualitative and quantitative evidences that we have succeeded in promoting the levels of all selected volatile compounds of black tea by the MeJA treatment. To the best of our knowledge, this is the first time to report the use of MeJA for improving the sensory quality of tea aroma. However, further investigation aimed at determining an accurate regulatory mechanism for MeJA treatment on tea aroma should be conducted for a better understanding of the overall process.

5 Conclusions

Significant differences in aroma components were found in black tea prepared from MeJA-treated tea plants. In general, the sensory quality of tea aroma was clearly improved. MeJA treatment could affect the expression of certain genes that modulate the relevant enzyme activity, resulting in increased free terpene alcohol aromas.

Compliance with ethics guidelines

Jiang SHI, Li WANG, Cheng-ying MA, Hai-peng LV, Zong-mao CHEN, and Zhi LIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要：

本文题目：茉莉酸甲酯诱导茶鲜叶制成的红茶中香气成分变化

Aroma changes of black tea prepared from methyl jasmonate treated tea plants

研究目的：为茉莉酸甲酯诱导来提高茶叶香气品质提供理论依据，为新型花香红茶的研制提供技术支持。

创新要点：首次将茉莉酸甲酯应用于诱导茶叶香气品质提高，初步验证了茶叶香气品质提高的本质原因：相关酶活性提高，基因表达上调。

研究方法：采用顶空固相微萃取法（HS-SPME）对红茶香气进行富集，气相色谱-质谱联用仪（GC-MS）进行解吸附分析，实时定量多聚酶链式反应（qRT-PCR）分析茶鲜叶中香气相关酶基因表达。

重要结论：茉莉酸甲酯诱导后的茶鲜叶中多酚氧化酶（PPO）活性上升，β-葡萄糖苷酶活性下降；PPO 和 β-樱草糖苷酶基因表达上调，β-葡萄糖苷酶基因表达下调。茉莉酸甲酯诱导后的茶鲜叶能明显提高由其制成的红茶香气品质，且萜烯醇类和萜烯类含量明显提高。

关键词组：红茶；香气；茉莉酸甲酯；顶空固相微萃取（HS-SPME）