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Dynamic Changes of the Total Content of Glycoside Aroma Components in Tobacco Leaves in Different Producing Areas During the Late Growth Period

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Abstract: In order to reveal the formation regularity of glycosidic latent aroma compounds and improve the aroma quality of tobacco leaves in the late growth stage, the dynamic changes of glycosidic aroma components and their mass fractions in tobacco leaves of the same variety in different producing areas (Neixiang and Baofeng in Henan Province; Yuxi and Puer in Yunnan Province; Huili in Sichuan Province) under the same cultivation conditions were analyzed by SDE-GC/MS. The results showed that in the late growing period of tobacco leaves: the composition of glycosylated aroma was the same (28 species) in tobacco leaves of 5 producing regions, including 4 aldehydes, 17 ketones, 5 alcohols, 1 lactone and 1 phenols; the total count of glycoside aroma components in tobacco leaves in 5 producing areas increased with the increase of tobacco maturity, which began to increase after 55 days of transplantion, reached the highest value at 85 days, and decreased slightly after 100 days; the total counts of glycoside aroma components of the tobacco leaves in Baofeng and Neixiang were higher than that in Huili; in Baofeng, Yuxi, Pu'er and Huili tobacco leaves, the contents of various glucoside aroma components were aldehydes > ketones > alcohols > others (lactone and phenols), while that in Neixiang was ketones > aldehydes > alcohols > others. At the late stage of tobacco growth, the change trend of the count of various glucoside aroma components was the same as that of the total count of glucoside aroma components.

Keywords: Tobacco Leaves, Glucoside Aroma Components, Count, Dynamic Changes

1. Introduction

The aroma of tobacco is the core content of tobacco leaf quality. The composition and content of tobacco aroma components are closely related to the quality and characteristics of tobacco leaves. The aroma components in tobacco exist in the form of free state and glycoside binding state [1, 2], and most of the aroma components in free state have strong volatility, which is easy to be volatilized in the process of high temperature processing. Glucoside is an acetal-type compound formed by dehydration of cyclic hemiacetal hydroxyl group of sugar or sugar derivatives with ROH, R_2NH and RSH, also known as a glycoside. When the ligand is aroma-causing component, the glycoside formed is a glycosidic latent aroma-causing substance [3], which is an important aroma-causing substance in tobacco [4]. Although it is not volatile or very low in natural state [5], it can be released during aging, processing or combustion. Therefore, its composition and content have an important effect on the aroma quality of tobacco leaves and flue gas [6].

In order to improve the aroma quality of tobacco leaves and increase the concentration of aroma, the non-volatile latent aroma compounds and their existing forms in tobacco have

been studied. It was found that most of them exist in the form of non-volatile glycosides [8, 10]. In the process of cigarette processing, the aroma components of free state and glycoside binding state also changed greatly [11, 12]. In recent years, the results of pyrolysis of glycosides and their application in cigarette flavoring have proved that glycosides can increase the stability of aroma release of tobacco leaves, make the aroma of tobacco leaves full and achieve a good effect of slow release of volatile aroma compounds [13, 17]. The higher the content of glycosides in tobacco leaves, the better the aroma of tobacco [18].

The formation and content of glycosides in tobacco were affected by many factors such as the varieties, parts of tobacco leaves and processing technology [19]. Through the analysis of four different types of tobacco under the same cultivation conditions, it was found that the content of glycosides in flue-cured tobacco was the highest, followed by sun-cured tobacco, Burley tobacco and perfume tobacco [20]. Some studies have also shown that the content of glacosidearoma components in different parts of tobacco leaves was different [21, 22]. By studying the important glycoside aroma substances in tobacco from 8 different producing areas with a unified cultivation method, it was found that there were obvious differences in the content of glyoside aromatic components such as benzyl alcohol, 3-hydroxydamarone in different producing areas [23]. During the process of tobacco leaf modulation, it was found that the total amount of glycoside aroma components decreased slightly from fresh tobacco leaves to yellowing stage, and decreased greatly after fixing color, and the amount after dry gluten was the least [24]. In addition, tobacco processing technology also had an effect on the total count of glycoside aroma components [25]. Up to now, few reports have been reported on the dynamic changes of the content of glycoside aroma components with maturity in growing tobacco leaves. Therefore, we analyzed the dynamic changes of the total count of glycoside aroma components with maturity of tobacco leaves at the late stage of tobacco growth under the same cultivation conditions with the same variety in different production areas, so as to provide the basis for increasing the total content of glycoside potential aroma substances in tobacco and improving the aroma quality of tobacco leaves and the exploitability of tobacco leaves.

2. Materials and Methods

2.1. Materials, Reagents and Instruments

The experimental flue-cured tobacco NC89 were selected from Neixiang and Baofeng in Henan Province, Yuxi and Pu'er in Henan Province and Huili in Sichuan Province with the same cultivation conditions. At 55 days after transplantion, the 18th leaves (5 cm) of one hundred tobacco plants were selected and marked. The 18th leaf was taken from 55, 70, 85 and 100 days after transplantion and brought back to the laboratory, respectively. Then the tobacco leaves were killed green at 80°C, dried at 105°C, ground into powder, and passed through a 245 μm mesh sieve, the experimental samples were gained and stored until needed.

Ethyl ether, anhydrous sodium sulfate (AR), dichloromethane, methanol, n-hexane, C_8~C_{20} series n-alkane standard samples (chromatographic purity, 99.9%) were purchased from China Pharmaceutical Group (Shanghai) Co., Ltd.; Amberlite XAD-2 macroporous resin, ethyl phenyl acetate (internal standard, > 99%), β-tamanone (98%), β-dihydrotamanone (95%), vanillin (97%), furfural (98%), 5-methylfurfural (>98%), benzaldehyde (99%), 2-acetylpyrrole (98%), 4-vinyl-2-metho- xyphenol (98%), β -ionone (96%), linalool (97%) were obtained from Sigma Aldrich Inc., USA; phenylethanol (97%), phenylacetaldehyde (95%) were purchased from Zhengzhou Alpha Chemical Co., Ltd; benzyl alcohol (96%), furfuryl alcohol (97%) were purchased from Shanghai Aladdin biochemical Technology Co., Ltd.; dihydrokiwifruit lactone (99%) was obtained from Guangzhou Daily Chemical Co., Ltd.); solanone (98%), 2-acetylfuran (99%), megastigmatrienone (98%, containing 4 isomers), farinoacetone (99%) were purchased from Pei Foo (Shanghai) Industrial Co., Ltd.

TRACE GC-DSQY MS Gas Chromatography-Mass Spectrometer (Thermo Fisher Scientific, USA); He gas (99.999%, Beijing Plaix practical Gas Co., Ltd.); Simultaneous distillation and extraction unit (customized); RE-52AA rotary evaporator was purchased from Shanghai Yarong biochemical instrument Factory.

2.2. Method

2.2.1. Separation of Glycoside Compounds

The killed green, dried and powdered tobacco sample (10. 0 g) was extracted by ultrasonic method for 1 hour in 100 mL methanol in conical bottle and the supernatant fluid was filtered. The residue was re-extracted by ultrasonic method for 1 hour in 80 mL methanol and the supernatant fluid was filtered. The two collected methanol extracts were combined and dried under vacuum.

The dry residue was suspended in 100 mL water and then was subjected to XAD-2 macroporous resin column chromatography eluting with 300 mL water to remove the acid, alkali and sugar, eluting with 250 mL ether-n-hexane (1: 1, v/v) to remove the free flavor substance and oil, waxy component, etc, and finally eluting with 350 mL methanol. The liquid methanol elution was collected and concentrated to driness in vacuum to obtain glycoside.

2.2.2. Qualitative and Quantitative Analysis of Aroma Components in Glycosides

The extract of glycosides was hydrolyzed with acidolysis [11] and then the aromatic components were extracted with simultaneous distillation and extraction method with a micro version apparatus: the gained glycoside (1.2.1) was dissolved in 100 mL purified water, then transferred to 500 mL flask, and finally 200 mL phosphate buffer (pH 2.5) was placed in this flask. 60 mL dichloromethane was placed in a 250 mL flask, which was placed in a 60°C water-bath. They were both distilled for 3.0 h at atmospheric pressure. At last, about

70 mL extract was obtained, dried with anhydrous sodium sulfate and transferred to a pear flask. 0.1 mL 0.104 8 mg/mL phenyl acetate (internal standard) dichloromethane solution was placed in the pear flask. The extract was condensed to 1.0 mL with a rotary evaporator. The resultant solution was fitered through a 0.45 μm PTFE filter for GC/MS analysis.

The analysis condition is:

The separation was achieved on fused silica capillary column DB-5-MS (30m×0.25 mm i.d.×0.25 μm d.f.). The injector and detector temperatures were 250°C and 280°C, respectively. The oven temperatures were programmed: 2min isothermal at 35°C and then increased at 20°C /min to 60°C and maintained for 2min; then from 60 to 180°C at 2°C /min and maintained for 2min; then from 180 to 280°C at 10°C /min and held for 20.0min. He was the carrier gas, column velocity was 0.8 mL/min and cylinder pressure was100 kPa. Mass spectra were scan at 70eV in the m/z range 50-650u.

Qualitative analysis: standard sample increment method combined with GC/MS was used for qualitative analysis of some glycoside aroma components with standard samples. The glycoside aroma components without standard samples can be searched by NIST02 and Wiley standard spectrum database and the matching degree can be preliminarily identified. The retention index (RI) was calculated by means of a series of n-alkanes, and compared with references [26, 34] for the secondary characterization of aroma components (Table 1, the retention index of aroma components determined by standard samples among them). The retention index of compounds is generally calculated by formula (1) under temperature programmed conditions [35]:

$$RI=100\times n+[100\times (t-t_n)/(t_{n+1}-t_n)] \quad (1)$$

In the formula : RI was the retention index of the sample; t is the retention time of the sample, min; t_n, t_{n+1} is the retention time of C_n and C_{n+1} n-alkane, respectively, $t_n<t<t_{n+1}$, min.

Qantitative determination: 22 glycoside aroma components with standard samples were measured by internal standard working curve. 22 spice monomers including β-damascenone, β-damascone and geranylacetone were prepared into a mixture of mother liquor with a concentration of about 0.6~16.3 mg/mL in dichloromethane. 1.0 mL of mixed mother liquor was accurately removed by pipette into a flask (100mL) and diluted with dichloromethane to 100 mL. 0.1, 0.5, 1.0, 1.5, 2.0, 3.0 mL mother liquor were taken by pipette into 6 flasks (10 mL), respectively; then 1.0 mL 0.104 8 mg/mL phenyl acetate (internal standard) dichloromethane solution was placed in each flask, respectively; and then dichloromethane was used to fix the volume. After GC analysis was performed, the standard working curve (Table 2) was obtained by regression analysis of the peak area ratio (x) of each standard sample by y. For components beyond the linear range, the sample can be diluted and then measured. For the six components without standard samples, the relative correction factor was assumed to be 1, and the internal standard method was adopted for quantification.

Table 1. *Qualitative results of glycoside aroma components identified by GC/MS in tobacco samples.*

No.	Aglycoside aroma components	Aroma characteristics	Retention index/RI①	
			RI_{exp}	RI_{lit}
1	Furfural	sweet, caramel	824	827[27]
2	3-Furanmethanol	scorched potato aroma	842	844[27]
3	2-Acetylfuran	flower, green fragrance	842	844[27]
4	5-Methylfurfural	fragrant, sweet	958	956[27]
5	Benzaldehyde	almonds, cherries aroma	956	957[27]
6	6-Methyl-5-hepten -2-one	gentle, delicate fragrance	977	992[29]
7	6-Methyl-5-hepten-2-ol	green, sweet aroma	990	992[34]
8	Benzyl alcohol	weak flower fragrance	1 028	1 030[28]
9	3,4-Dimethyl-2,5-furandione		950	951[32]
10	Benzeneacetaldehyde	flower, soap fragrance	1 042	1 039[28]
11	2-Acetylpyrrole	sweet, flower fragrance	1 060	1 057[27]
12	Linalool	floral aroma, lemon aroma	1 098	1 100[31]
13	Phenylethyl alcohol	sweet aroman, nutty aroma	1 106	1 108[27]
14	Isophorone oxide	sweetening	1 098	1 111[32]
15	2-Methoxy-4-vinylphenol	bitterness	1 322	1 315[31
16	β-Damascone	floral aroma	1 410	1 412[30]
17	Solanone	fragrant and sweet	1 355	1 356[27]
18	β-Damascenone	delicate fragrance rose fragrance	1 399	1 401[27]
19	Geranylacetone	green incense	1 450	1 453[28]
20	β-Ionone	sweet aroma, woody fragrance	1 488	1 485[29]
21	Dihydroactinidiolide	slight cool feeling	1 510	1 513[27]
22	Megastigmatrienone 1	refreshing, sweet grass	1 550	1 549[27]
23	Megastigmatrienone 2	delicate fragrance, sweet	1 567	1 567[27]
24	Megastigmatrienone 3	faint scent, fragrant and sweet	1 596	1 598[27]
25	3-Hydroxy-β-damascone		1 611	1 601[27]
26	Megastigmatrienone 4	faint scent, fragrant and sweet	1 609	1 612[27]
27	β-Solavetivone	woody fragrance	1 426	
28	Farinoacetone		1 910	1 904[27]

Note: RI_{exp} is the retention index (DB-5 column) of the compounds calculated from the experimental results. RI_{lit} is the retention index of the corresponding compounds in the literature (DB-5 column or column with the same stationary phase as DB-5).

Table 2. *The standard working curves of aroma components and linear range.*

Aroma components	Standard working curve	R^2	Linear range/(μg·mL^{-1})	Detection limit/(μg·mL^{-1})
Geranylacetone	y=8.728x+0.001	0.998 7	0.10~3.24	0.05
β-Damascenone	y=8.866x−0.072	0.999 3	0.72~21.70	0.11
β-Damascone	y=8.558x+0.100	0.998 4	0.28~8.44	0.08
Megastigmatrienone 1	y=8.708x+0.084	0.996 8	0.31~9.38	0.06
Megastigmatrienone 2	y=8.744x+0.001	0.999 9	0.60~18.04	0.13
Megastigmatrienone 3	y=8.735x+0.001	0.998 2	0.30~9.01	0.08
Megastigmatrienone 4	y=8.751x+0.013	0.992 5	0.42~12.76	0.10
Solanone	y=8.730x+0.062	0.999 3	1.12~33.71	0.06
Farinoacetone	y=8.759x+0.013	0.998 6	0.40~12.03	0.09
Benzaldehyde	y=8.828x+0.034	0.999 8	0.82~24.64	0.07
Benzeneacetaldehyde	y=8.723x+0.028	0.999 2	0.78~23.43	0.12
Furfural	y=8.808x+0.015	0.999 5	1.61~48.85	0.09
5-Methylfurfural	y=8.707x+0.179	0.993 5	0.61~18.31	0.08
Benzyl alcohol	y=8.683x+0.053	1.000 0	0.67~20.11	0.04
Linalool	y=7.893x+0.181	0.995 1	0.28~8.44	0.07
3-Furanmethanol	y=8.787x−0.048	0.999 7	0.50~15.06	0.15
Phenylethyl alcohol	y=8.718x+0.010	0.999 9	0.08~2.43	0.03
2-Acetylfuran	y=8.633x−0.020	0.998 7	0.08~2.42	0.02
Dihydroactinidiolide	y=8.785x+0.018	0.996 9	0.07~2.34	0.04
2-Acetylpyrrole	y=8.533x+0.002	0.999 7	0.07~2.16	0.05
β-Ionone	y=8.744x+0.001	0.999 8	0.08~2.56	0.04
2-Methoxy-4-vinylphenol	y=8.958x−0.009	0.998 5	0.06~1.86	0.02

3. Results and Analysis

3.1. Glycoside Aromatic Components and Mass Fraction of Main Aroma Components

Glycosides are secondary metabolites of tobacco leaves, the content is low, the species is large, and it is very close to oligosaccharides, pectin, amino acids, pigments and other substances, so it is difficult to obtain a complete standard sample [12]. Glycoside potential aroma compounds extracted from tobacco leaves were hydrolyzed under acidic conditions, and the glycoside aroma components in NC89 tobacco leaves from five different production areas were analyzed by GC/MS method. According to the GC-MS results, 28 main glycoside aroma components were identified (Table 1), among which there are 17 ketones, 4 aldehydes, 5 alcohols, 1 lactones and 1 phenols. The results showed that the glycoside aroma components were the same in tobacco leaves of the same cultivar under the same cultivation conditions, but the content of each component was very different due to the different ecological environment. Among them, the 16 main glycoside aroma components which have great influence on the aroma of tobacco leaves or have a relatively high mass fraction are: geranylacetone, β-damascenone, β-damascone, megastigmatrienone (1, 2, 3, 4), solanone, farnesylacetone, furfural, benzaldehyde, benzeneacetaldehyde, 5-methylfurfural, benzyl alcohol, linalool and 3-furanmethanol, the dynamic changes of their mass fraction were shown in Table 3.

Table 3. *The contents of main aglycoside aroma components of tobacco leaves in different producing areas at the late growth stage (μg g^{-1}).*

Aroma components	Time after transplantion/d	Neixiang	Baofeng	Yuxi	Huili	Pu´er
Geranylacetone	55	0.99±0.06	0.26±0.03	0.56±0.02	0.38±0.03	0.18±0.04
	70	1.52±0.13	0.28±0.02	0.67±0.08	1.12±0.07	0.21±0.03
	85	1.68±0.14	0.35±0.04	0.84±0.03	1.66±0.05	0.53±0.05
	100	1.72±0.05	0.30±0.06	0.71±0.04	1.52±0.06	0.48±0.06
β-Damascenone	55	8.92±0.13	3.56±0.05	3.12±0.07	4.05±0.05	1.88±0.02
	70	11.12±0.09	5.32±0.03	4.03±0.02	6.78±0.03	3.21±0.06
	85	12.43±0.12	7.34±0.12	4.56±0.12	12.03±0.14	5.53±0.05
	100	11.63±0.14	5.65±0.06	4.34±0.07	7.65±0.07	5.25±0.12
β-Damascone	55	1.56±0.05	0.83±0.05	0.68±0.05	1.42±0.06	0.45±0.02
	70	1.67±0.04	1.45±0.04	0.86±0.04	2.26±0.08	0.78±0.04
	85	2.02±0.06	2.68±0.12	1.06±0.03	3.78±0.12	2.34±0.12
	100	1.87±0.07	2.36±0.07	0.96±0.07	3.34±0.04	2.16±0.06
Megastigmatrienone 1	55	1.54±0.05	2.12±0.06	1.35±0.03	0.78±0.03	0.57±0.03
	70	2.78±0.13	3.87±0.14	2.51±0.21	1.67±0.06	1.46±0.08
	85	3.24±0.09	6.24±0.17	3.87±0.13	2.83±0.12	5.72±0.14
	100	2.96±0.06	5.83±0.12	3.45±0.09	2.68±0.07	4.54±0.12
Megastigmatrienone 2	55	3.86±0.13	2.34±0.08	0.88±0.03	2.87±0.07	0.95±0.07
	70	4.56±0.09	5.23±0.06	2.35±0.13	4.89±0.19	3.28±0.17
	85	5.47±0.13	5.86±0.08	3.42±0.16	8.36±0.18	6.78±0.18
	100	4.82±0.15	4.76±0.16	2.98±0.06	7.62±0.14	6.33±0.21

Aroma components	Time after transplantion/d	Neixiang	Baofeng	Yuxi	Huili	Pu´er
Megastigmatrienone 3	55	1.26±0.09	0.89±0.07	0.63±0.05	0.86±0.07	0.78±0.06
	70	2.52±0.06	1.24±0.09	0.97±0.12	1.45±0.06	1.32±0.07
	85	2.88±0.11	2.36±0.05	1.76±0.08	2.63±0.12	2.67±0.02
	100	2.73±0.09	2.15±0.13	1.44±0.05	2.32±0.09	2.44±0.13
Megastigmatrienone 4	55	1.96±0.06	1.68±0.11	0.83±0.06	2.98±0.08	1.85±0.09
	70	3.24±0.12	4.52±0.16	1.96±0.12	5.67±0.14	3.87±0.16
	85	6.03±0.16	5.68±0.04	3.38±0.06	10.66±0.21	9.34±0.17
	100	5.84±0.18	4.89±0.14	2.56±0.11	7.86±0.24	8.25±0.22
Solanone	55	28.97±1.23	22.32±0.98	15.67±0.76	18.23±0.66	12.86±0.68
	70	35.32±1.07	29.19±1.24	19.78±1.02	21.54±0.88	17.24±1.12
	85	40.13±1.56	37.86±1.05	27.83±0.86	30.56±1.05	22.45±0.96
	100	37.78±0.09	34.55±0.99	23.43±1.23	25.74±0.96	19.63±0.85
Farinoacetone	55	2.46±0.13	1.43±0.05	1.21±0.05	1.98±0.09	0.89±0.08
	70	3.56±0.21	2.56±0.08	1.67±0.08	3.36±0.12	1.26±0.06
	85	4.32±0.09	3.24±0.13	2.45±0.09	4.13±0.08	1.97±0.08
	100	3.77±0.15	2.89±0.09	1.87±0.06	3.67±0.14	1.76±0.11
Benzaldehyde	55	2.99±0.12	2.36±0.07	1.98±0.08	3.87±0.22	3.24±0.12
	70	3.66±0.13	4.34±0.12	3.13±0.09	4.35±0.09	5.76±0.19
	85	7.67±0.17	5.76±0.04	5.96±0.16	6.67±0.23	8.72±0.34
	100	5.65±0.21	4.13±0.15	5.32±0.18	5.87±0.5	7.65±0.21
Benzeneacetaldehyde	55	3.23±0.13	3.89±0.16	1.89±0.07	3.82±0.09	3.62±0.09
	70	4.47±0.09	4.59±0.14	3.24±0.22	4.13±0.07	4.02±0.08
	85	4.96±0.17	6.13±0.19	4.26±0.13	4.76±0.17	5.36±0.21
	100	4.74±0.21	5.23±0.21	3.87±0.16	4.51±0.23	4.82±0.13
Furfural	55	36.24±0.34	55.46±0.87	33.45±0.98	51.23±0.65	27.86±0.88
	70	42.65±0.89	60.13±0.49	62.23±0.78	60.68±0.23	49.35±0.76
	85	59.13±0.76	67.88±0.46	70.47±1.04	66.12±0.54	64.54±1.03
	100	50.67±0.85	64.27±0.56	68.09±0.84	63.21±0.89	53.68±1.24
5-Methylfurfural	55	2.24±0.08	0.76±0.07	0.56±0.07	2.98±0.17	0.76±0.04
	70	2.87±0.09	1.87±0.13	1.23±0.05	3.56±0.14	1.54±0.06
	85	3.65±0.16	2.68±0.09	1.86±0.07	4.69±0.06	2.23±0.08
	100	3.23±0.06	2.35±0.08	1.53±0.03	3.84±0.19	1.87±0.15
Benzyl alcohol	55	2.06±0.04	2.56±0.04	2.38±0.07	1.65±0.06	0.96±0.05
	70	5.48±0.13	6.83±0.15	3.46±0.15	2.46±0.09	1.76±0.04
	85	7.86±0.23	8.54±0.21	4.67±0.09	4.78±0.20	4.35±0.08
	100	5.77±0.19	7.69±0.18	3.76±0.06	3.54±0.06	3.23±0.17
Linalool	55	2.13±0.08	3.87±0.12	2.37±0.09	2.24±0.07	1.78±0.06
	70	3.24±0.15	4.35±0.09	2.96±0.12	2.71±0.08	2.57±0.12
	85	6.58±0.17	5.23±0.16	4.15±0.16	3.45±0.11	2.97±0.05
	100	5.32±0.21	4.66±0.08	3.23±0.19	3.03±0.06	2.82±0.06
3-Furanmethanol	55	5.17±0.22	4.67±0.23	1.68±0.05	2.64±0.12	2.03±0.17
	70	6.09±0.13	4.98±0.05	1.89±0.12	3.25±0.21	2.45±0.08
	85	7.02±0.25	5.43±0.16	2.35±0.07	5.89±0.13	3.27±0.09
	100	6.43±0.10	5.01±0.13	2.14±0.09	4.69±0.24	2.98±0.14

3.2. Dynamic Change Analysis of the Total Content of the Glycoside Aroma Components in Tobacco Leaves from Different Production Areas

3.2.1. Dynamic Changes of the Total Content of the Glycoside Aroma Components in Tobacco Leaves

It can be seen from figure 1 that the total contents of glycoside aroma components in the five producing areas were different in the same period, but had similar dynamic change trend which the total content increases with the maturity. After 55 days of transplantion, the total content of glycoside aroma components in tobacco leaves showed an increasing trend, reaching the maximum at 85 days, and then decreased slightly at 100 days. There were significant differences in total amount between different periods in the same producing area. After 55 days of transplantion, the total count of glycoside aroma components of tobacco leaves in five producing areas was Baofeng > Neixiang > Huili > Yuxi > Pu'er, 70 days later, the order of total aroma components was Baofeng > Neixiang > Pu'er, Huili > Yuxi, 85 days later the order was Neixiang > Baofeng > Huili > Pu'er > Yuxi > Pu'er, 100 days later, the order was: Neixiang > Baofeng > Huili > Yuxi > Pu'er. In general, the total aroma components of tobacco leaves from Baofeng and Neixiang in Henan province were relatively high during the late period of tobacco growth.

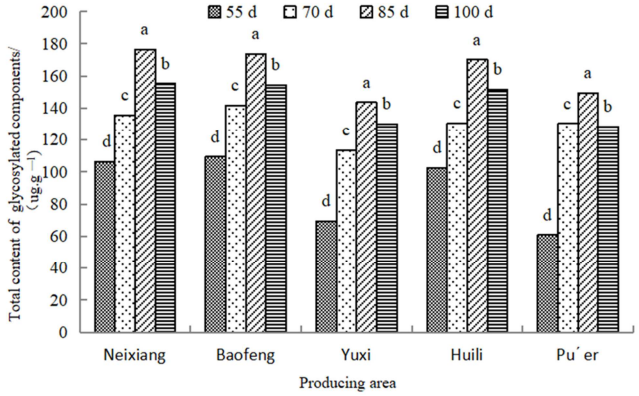

Figure 1. *Dynamic changes of the total contents of glycoside aroma components in tobacco leaves from different prodcing areas during the late period of growth.*

3.2.2. Dynamic Changes of the Content of Glycosyl Ketones in Tobacco Leaves

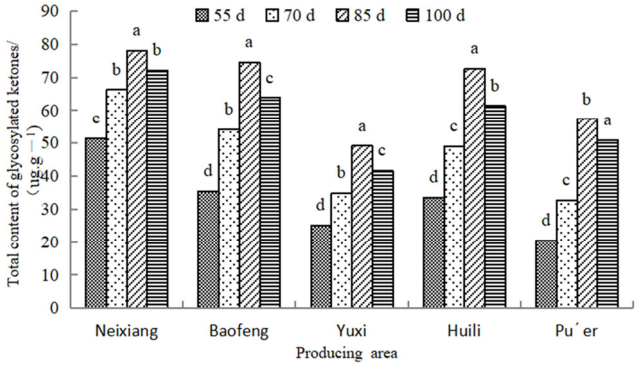

Figure 2. *Dynamic changes of the content of glycosyl ketones in tobacco leaves from different producing areas during the late period of growth.*

It can be seen from Figure 2 that the content of glycosyl ketones in tobacco leaves was increasing as a whole. The change trend of mass fraction of glycosyl ketones in tobacco leaves from five producing regions was the same as that of the total glycoside aroma components. From tables 1 and 2, we found that 17 glycosyl ketones were identified, among which the mass fraction of solanone, megastigmatrienone (1,2,4) and β-damascenone are higher relatively. Their change trends of mass fraction were the same as that of total glycosyl ketones during the late growth period. After 55 days and 70 days of transplantion, the order of the mass fraction of glycosyl ketones was Neixiang > Baofeng > Huili > Yuxi > Puer; 85 and 100 days later, it was Neixiang > Baofeng > Huili > Pu'er > Yuxi. Additionally, the percentage of glycosyl ketones in the total amount of glycosyl aroma components was: the range of Neixiang tobacco leaves from 44.8% to 48.8%, that of Baofeng tobacco leaves from 32.4% to 41.2%, that of Yuxi tobacco leaves from 30.7% to 36%, that of Huili tobacco leaves from 32.8% to 44%, that of Pu'er tobacco leaves from 25% to 39.6%. The mass fraction of glycosyl ketones of tobacco leaves in Neixiang was higher relatively.

3.2.3. Dynamic Changes of the Content of Glycosyl Aldehydes in Tobacco Leaves

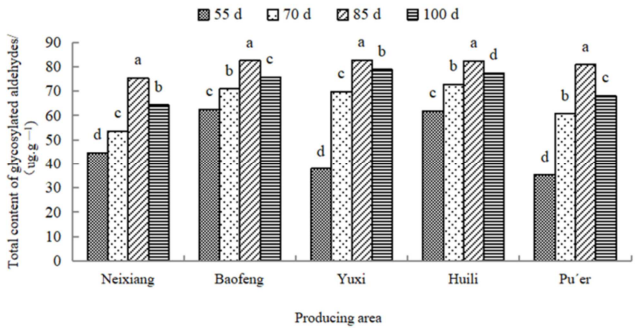

Figure 3. *Dynamic changes of the content of glycosyl aldehydes in tobacco leaves from different producing areas during the later period of growth.*

As the results shown in Figure 3 and Table 2, four glycosyl aldehydes were identified, among which the mass fraction of furfural was the highest. The change trend of the content of glycosyl aldehydes with maturity of tobacco leaves in the five producing regions was the same as that of the total content of glycoside aroma components in tobacco leaves. 55 days after transplantion, the order of the content of glycosyl aldehydes in tobacco leaves in different producing areas was Baofeng > Huili > Neixiang > Yuxi > Pu'er; 70 days later, it was Huili > Baofeng > Yuxi > Pu'er > Neixiang; 85 days later, it was Yuxi, Huili and Baofeng > Neixiang > Pu'er ; 100 days later, it was Yuxi > Huili > Baofeng > Neixiang > Pu'er. The percentage of glycosyl aldehydes in the total amount of glycoside aroma components was: the range of Neixiang tobacco leaves from 39.5% to 42.7%, that of Baofeng tobacco leaves from 47.4% to 57.1%, that of Yuxi tobacco leaves from 54.4% to 61.5%, that of Huili tobacco leaves from 47.2% to 60.5%, that of Pu 'er tobacco leaves from 46.5% to 58.2%. The percentage of glycosyl aldehydes in the total amount of glycoside aroma components was higher in Baofeng, Yuxi, Pu 'er and Huili tobacco leaves than that of glycosyl ketones in the same period, while the percentage of glycosyl aldehydes in Neixiang tobacco leaves was lower than that of glycosyl ketones.

3.2.4. Dynamic Changes of the Content of Glycosyl Alcohols in Tobacco Leaves

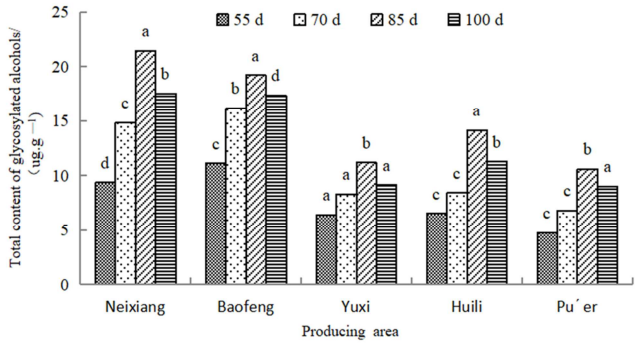

Figure 4. *Dynamic changes of the content of glycosyl alcohols in tobacco leaves from different producing areas during the later period of growth.*

As the results shown in Figure 4 and Table 2, five glycosyl alcohols were identified in tobacco samples, among which the mass fraction of benzyl alcohol, linalool and furfuryl alcohol was relatively high (table 2). The change trend of the content of glycosyl alcohols in tobacco leaves in five producing areas was the same as that of the total content of the glycoside aroma components. After 55 and 70 days of transplantion, the order of the mass fraction of glycosyl alcohols in tobacco leaves in different producing areas was Baofeng > Neixiang > Huili > Yuxi > Pu'er, while 85 days and 100 days later, it was Neixiang > Baofeng > Huili > Yuxi > Pu'er. The percentage of glucosyl alcohols in the total amount of glycoside aroma components was: the range of Neixiang tobacco leaves from 8.8% to 12.2%, the range of Baofeng tobacco leaves from 10.1% to 11.4%, the range of Yuxi tobacco leaves from 7.0% to 9.2%, the range of Huili tobacco leaves from 6.4% to 8.1%, the range of Pu'er tobacco leaves from 5.2% to 7.8%. All of the percentage of glycosyl alcohols was lower than that of glycosyl ketones and glycosyl aldehydes in the same period.

3.2.5. Dynamic Changes of the Content of Other Glycosyl Components in Tobacco Leaves

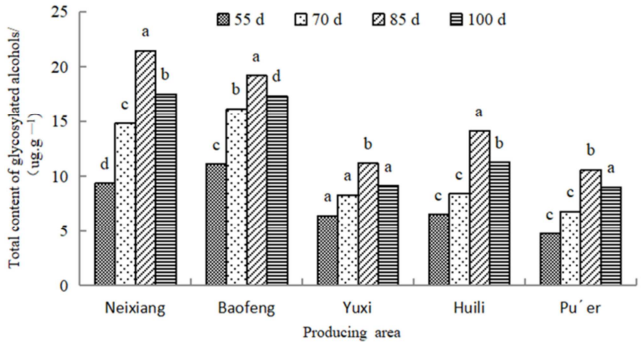

Figure 5. *Dynamic changes of the content of other glycosyl components in tobacco leaves from different producing areas during the late period of growth.*

As the results shown in Figure5 and Table 2, two components, dihydroactinidiolide and 4-vinyl-2-methoxyphenol, were also identified in tobacco samples, whose content accounted for less than 1% of the total content of glycoside aroma components in tobacco. The change trend of the mass fraction of the two components with maturity of tobacco leaves in the five producing regions was the same as that of the total content of glycoside aroma components in tobacco. After 55 days of transplantion, the order of the mass fraction of the two glycosyl components in tobacco leaves in different producing areas was Neixiang > Baofeng > Huili > Yuxi > Pu'er; 70, 85 and 100 days later, it was Neixiang> Huili, which was higher than Yuxi, Baofeng and Pu'er.

4. Summary and Discussion

According to the results shown in part 3 (3. Results and analysis), under the same cultivar and the same cultivation conditions, the glycoside aroma components of tobacco leaves in five different growing areas during the late period of growth were the same. 28 species of the glycoside aroma components were detected, including 17 glycosyl ketones, 4 glycosyl aldehydes, 5 glycosyl alcohols, 1 glycosyl lactone and 1 glycosyl phenols.

The chemical composition of tobacco leaves was determined by genetic factors, and the content of each component was affected by environmental factors. Therefore, the content of each glycoside aroma component in different producing areas were different in the same period. However, the change trend of the total content of glucoside aroma components was the same with the maturity of tobacco leaves, that is, the total content increased after 55 days of transplantion, reached the highest value after 85 days of transplantion, and then decreased slightly after 100 days of transplantion. This may be due to the fact that, with the increase of tobacco leaf maturity, some glycoside latent aroma compounds were first in the formation and accumulation state, and some of them were hydrolyzed by endogenous enzymes in the near maturity stage[36]. There was a significant difference in the total amount of tobacco glycoside aroma components in different growth stages in the same producing area.

Recently, it was found that the glycoside latent aroma substance had a great effect on the formation of tobacco luzhou-flavor style [18]. The luzhou-flavor style of tobacco leaves in henan province has been somewhat weakened, and measures to strengthen tobacco luzhou-flavor style can be studied from the point of view of glycoside latent aroma substance. The results in the study showed that the counts of glycoside aroma components of Neixiang and Baofeng tobacco leaves in Henan Province were higher than that of Yuxi, Pu'er tobacco leaves in Yunan Province, and that of Huili tobacco leaves in Sichuan Province, which might be related to the luzhou-flavor type of tobacco leaves in Henan producing areas. The results in this paper can provide the basis for further strengthening the tobacco flavor research.

So far, the improvement of tobacco aroma quality by using glucoside latent aroma compounds is only limited to the study of the stability of some glucoside monomers, thermal cracking and cigarette adding incense. The research on how to improve the content of glycosides in tobacco leaves is rarely involved. In this paper, we try to start with the formation of glycosides, revealing the regularity of the formation of glycoside latent aroma substance content in tobacco leaves at the late stage of growth. It laid a foundation for further research on how to increase the content of the glycoside latent substances so order to improve the quality of tobacco leaves.

5. Conclusion

In this paper, we analyzed the dynamic changes of the content of glycoside aroma components in tobacco leaves with the same variety in the same cultivation condition during the late growth period in five producing areas. The results showed that: (1) the glycoside aroma components identified in tobacco leaves in five producing areas were the same, but the mass fractions of each component were different; (2) the

dynamic change trend of the total amount of glycosidic aroma components in tobacco leaves in five producing areas with tobacco maturity was the same; the dynamic change trend of different glycosyl aroma components with tobacco maturity was the same as that of total glycoside aroma components; (3) during the late growth period of tobacco leaves, the total content of glycoside aroma components in Baofeng and Neixiang tobacco leaves was higher than that in Huili, Yuxi and Pu'er tobacco leaves.

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