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Relationship between Free Amino Acid Index and Burnt-Mellow-Sweet Aroma Style Intensity of Flue-Cured Tobacco Leaves

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Abstract

80 samples of flue-cured tobacco leaves in Ganzhou area, Jiangxi Province in China were selected, relationships between free amino acid index and burnt-mellow-sweet aroma style intensity of leaves were studied with correlation analysis and stepwise regression analysis. In contrast to the content of Trp, Ile, Ser, Cys, β-Ala, Lys and the ratio of β-AiBA/TFAA, the content of Asp, Ala, Gly, Leu, β-AiBA and the ratio of Trp/TFAA, Ile/TFAA, Val/TFAA, Thr/TFAA, Ser/TFAA, Cys/TFAA, Asn/TFAA, β-Ala/TFAA, Lys/TFAA and Pro/TFAA in flue-cured tobacco leaves had extremely significant positive correlation with burnt-mellow-sweet aroma style intensity. There was a significant negative correlation between burnt-mellow-sweet aroma style intensity and the content of Phe, Tyr, Asn, γ-ABA. 31 free amino acid indexes including the content of Asp, Glu, Phe etc. and the ratio of Glu/TFAA, Phe/TFAA, Trp/TFAA, etc. were main free amino acid index influencing burnt-mellow-sweet aroma style intensity of flue-cured tobacco leaves, which jointly decides 99.1% of variation of burnt-mellow-sweet aroma style intensity.

Keywords: Flue-cured tobacco; Free amino acid index; Burnt-mellow-sweet aroma style intensity; Correlation analysis; Stepwise regression analysis

Introduction

Determination of mainly affecting material index for the intensity of burnt-mellow-sweet aroma style of flue-cued tobacco leaves is the key and basis of material character and prediction of the characteristics of burnt-mellow-sweet aroma style of flue-cured tobacco leaves in china [1-3]. Qiao et al. established the expert sensory evaluation method of burnt-mellow-sweet aroma style of flue-cured tobacco leaves [4,5]; Wang et al. and Luo et al identified the characteristics of the conventional chemical composition, alkaloid, polyphenol, organic acid and aromatic composition of burnt-mellow-sweet aroma style strength of flue-cured tobacco leaves. The free amino acids are one of the main aromas sources in flue-cured tobacco leaves, the non-enzyme browning reaction between amino acids and reduced sugars in baking and aging process is an important aromatic reaction, which can form pyridoxine, pyrazine and furan compounds with important effects on tobacco aromas, and some amino acids (such as phenylalanine) can also be directly broken down into aromatic substances including benzoyl alcohol and phenyl ethanol [6,7]. Most of previous studies have focused on the relationship between the free amino acid indexs and external quality of flue-cured tobacco leaves [8-10], the releasing amount of harmful substances, the aroma quality, and the relationship between flavor type [10,11].

The relationship between the free amino acid index of flue-cured tobacco leaves and burnt-mellow-sweet aroma style intensity has not been reported. At present, the relationship between the free amino acid index of flue-cured tobacco leaves and the intensity of burnt-mellow-sweet aroma style has not been reported. It takes 80 samples of flue-cured tobacco leaves in Ganzhou, Jiangxi province as the material in this paper, relevant analysis, gradual regression analysis and other statistical methods were used to study the relationship between the free amino acid index of tobacco leaves and burnt-mellow-sweet aroma style intensity.

The study will clarify the free amino acids of flue-cured tobacco leaves and screen out main free amino acid index affecting the sweet aroma strength of tobacco leaves, which provide the theoretical basis for the characterization and char-sweet prediction of burnt-mellow-sweet aroma style intensity through the free amino acid index of flue-cured tobacco leaves.

Materials and Methods

Materials

80 flue-cured tobacco leaves samples with C3F and B2F grade were collected from Ganzhou in Jiangxi Province. It were collected from Ganzhou tobacco-planting area of 40 different villages, 4.0kg for each tobacco leaf sample. Each cigarette sample is randomly divided into 2 parts: 1.5kg sample were crushed and screened with 40 mesh for free amino acid content determination. And other 2.5kg samples was rolled into cigarette with 70mm length, 24.5mm circumferences, which used for sensory evaluation of burnt-mellow-sweet aroma style intensity after environment balance with a temperature (22±1) and relative humidity (60±2 %) for more than 48h.

Measuring items and methods

L-8900 amino acid automatic analyzer were used to determine the content of free amino acid (mass fraction), after processing the tobacco leaf sample according to the method of literature [12], the content of free amino acid (mass fraction) was determined by the

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Table 1: Description statistics on the free amino acid index of tobacco leaves.

Indicator	Min	Max	Average	Standard Deviation	Variation Factor (%)
Asp (mg/g)	0.03	0.11	0.06	0.02	29.04
Glu (mg/g)	0.01	0.11	0.06	0.03	46.91
Phe (mg/g)	0.01	0.08	0.04	0.02	41.48
Trp(mg/g)	0.01	0.07	0.02	0.01	52.35
Tyr (mg/g)	0.01	0.02	0.01	0	35.3
Ala (mg/g)	0.06	0.21	0.13	0.03	25.81
Gly (mg/g)	0.01	0.02	0.01	0	25.14
Ile(mg/g)	0.01	0.05	0.03	0.01	40.14
Leu (mg/g)	0.01	0.03	0.02	0.01	34.31
Val (mg/g)	0.17	0.39	0.26	0.05	20.22
Thr (mg/g)	0.06	0.17	0.1	0.03	25.43
Ser (mg/g)	0.01	0.08	0.03	0.02	75.74
Cys (mg/g)	0.06	0.32	0.19	0.08	40.52
Asn (mg/g)	0.11	0.67	0.33	0.13	39.57
β-Ala (mg/g)	0.05	0.3	0.16	0.07	43.64
β-AiBA (mg/g)	0.01	0.04	0.02	0.01	58.4
γ- ABA (mg/g)	0.01	0.05	0.03	0.01	35.55
Lys (mg/g)	0.01	0.02	0.01	0	16.11
His (mg/g)	0.01	0.04	0.02	0.01	42.49
Arg (mg/g)	0.01	0.02	0.01	0	19.36
Pro (mg/g)	1.03	4.48	2.47	0.87	35.36
Total free amino acids (mg/g)	2.03	6.77	4.01	1.19	29.71
Asp ratio (%)	0.76	2.64	1.6	0.39	24.2
Glu ratio (%)	0.43	2.65	1.4	0.55	38.84
Phe ratio (%)	0.46	1.46	0.94	0.26	27.5
Trp ratio (%)	0.29	1.21	0.59	0.21	35.27
Tyr ratio (%)	0.18	0.61	0.34	0.11	31.27
Ala ratio (%)	2.21	4.98	3.36	0.53	15.67
Gly ratio (%)	0.18	0.6	0.37	0.08	22.88
Ile ratio (%)	0.36	1.18	0.71	0.21	30.18
Leu ratio (%)	0.19	0.64	0.39	0.11	27.13
Val ratio (%)	2.92	11.82	6.93	1.77	25.48
Thr ratio (%)	1.61	4.36	2.7	0.59	22.05
Ser ratio (%)	0.16	2.83	0.79	0.69	87.05
Cys ratio (%)	2.13	7.95	4.81	1.48	30.81
Asn ratio (%)	3.77	13.27	8.33	2.43	29.19
β-Ala ratio (%)	2.25	7.92	3.96	1.47	37
β-AiBA ratio (%)	0.12	0.91	0.38	0.2	52.5
γ- ABA ratio (%)	0.42	1.31	0.74	0.2	27.33
Lys ratio (%)	0.12	0.41	0.22	0.08	35.28
His ratio (%)	0.19	0.61	0.39	0.09	22.17
Arg ratio (%)	0.12	0.47	0.22	0.08	36.16
Pro ratio (%)	44.51	72.1	60.8	6.43	10.58

method of literature [13]. According to the method of literature [14], the evaluation team composed of 7 members of national evaluation and suction committee conducted a sensory evaluation of burnt-mellow-sweet aroma style intensity of flue-cured tobacco leaves, 5-point scale was used, the higher the score, the more obvious the intensity.

Data analysis

SPSS19.0 statistical software was used to perform the statistical description, correlation analysis and gradual regression analysis for free amino acids in flue-cured tobacco leaves and the sensory evaluation data of burnt-mellow-sweet aroma style intensity.

Results and Analysis

Statistical description of free amino acid index of flue-cured tobacco leaves

The statistical characteristics of free amino acid index of flue-cured tobacco leaves can be found in Table 1. The content of Proline (Pro) in flue-cured tobacco leaves is significantly higher than that of other types of free amino acids, accounting for more than 44.5% of the total free amino acid content. Tyr, Gly, Lys and Arg content are relatively low, as a percentage of total free amino acid content (Tyr/TFAA, Gly/TFAA, Lys/TFAA, Arg/TFAA) were all below 0.7%. The content of Ser, β-AiBA and the proportion of its to total free amino acid content (Ser/TFAA, β-AiBA/TFAA) have relatively large variations, with the coefficient of variation being greater than 51.5%; The variation of Arg, Lys, Ala/TFAA and Pro/TFAA was relatively low, with an average variation of less than 19.5%.

Correlation analysis between the free amino acid index of flue-cured tobacco leaves and burnt-mellow-sweet aroma style intensity

The correlation between the free amino acid index of flue-cured tobacco leaves and burnt-mellow-sweet aroma style intensity was seen in Table 2. The content of Asp, Ala, Gly, Leu, β-AiBA, His and the proportion of Trp/TFAA, Ile/TFAA, Val/TFAA, Thr/TFAA, Ser/TFAA, Cys/TFAA, Asp/TFAA, β-Ala/TFAA, Lys/TFAA and Pro/TFAA were all positively correlated with burnt-mellow-sweet aroma style intensity. The content of Glu, Pro and the proportion of Glu/TFAA and Phe/TFAA were significantly correlated with burnt-mellow-sweet aroma style intensity.

The content of Trp, Ile, Ser, Cys, β-Ala, Lys and the ratio of β-AiBA/TFAA was significantly negatively correlated with burnt-mellow-sweet aroma style intensity. And the content of Phe, Tyr, Asn, γ-ABA was significantly negatively correlated with burnt-mellow-sweet aroma style intensity. The content of Val, Thr, Arg, TFAA and Asp/TFAA, Tyr/TFAA, Ala/TFAA, Gly/TFAA, Leu/TFAA, γ-ABA/TFAA, His/TFAA, Arg/TFAA were not significantly uncorrelated to burnt-mellow-sweet aroma style intensity in flue-cured tobacco leaves.

Stepwise regression analysis of free amino acid index and burnt-mellow-sweet aroma style intensity of flue-cured tobacco leaves

The widely used multiple regression analysis was mainly focused on the relationship between several independent variables and a dependent variable analysis method. The "optimal" regression model

Table 2: Correlation between the free amino acid index of flue-cured tobacco leaves and burnt-mellow-sweet aroma style intensity.

Indicator	Correlation coefficients	P value	The level of significantness
Asp (mg/g)	0.893	0.001	**
Glu (mg/g)	0.822	0.017	*
Phe (mg/g)	-0.845	0.01	*
Trp (mg/g)	-0.914	0	**
Tyr (mg/g)	-0.754	0.046	*
Ala (mg/g)	0.911	0	**
Gly (mg/g)	0.888	0.002	**
Ile (mg/g)	-0.91	0	**
Leu (mg/g)	0.878	0.003	**
Val (mg/g)	-0.274	0.627	-
Thr (mg/g)	-0.442	0.413	-
Ser (mg/g)	-0.912	0	**
Cys (mg/g)	-0.847	0.009	**
Asn (mg/g)	-0.828	0.015	*
β-Ala (mg/g)	-0.877	0.003	**
β-AiBA (mg/g)	0.906	0	**
γ- ABA (mg/g)	-0.78	0.033	**
Lys (mg/g)	-0.906	0	**
His (mg/g)	0.915	0	**
Arg (mg/g)	-0.003	0.998	-
Pro (mg/g)	0.83	0.014	*
Total free amino acids (mg/g)	-0.005	0.993	-
Asp ratio (%)	-0.53	0.232	-
Glu ratio (%)	0.793	0.027	*
Phe ratio (%)	0.816	0.018	*
Trp ratio (%)	0.9	0.001	**
Tyr ratio (%)	0.739	0.054	-
Ala ratio (%)	-0.264	0.64	-
Gly ratio (%)	0.04	0.935	-
Ile ratio (%)	0.893	0.001	**
Leu ratio (%)	-0.137	0.811	-
Val ratio (%)	0.909	0	**
Thr ratio (%)	0.879	0.003	**
Ser ratio (%)	0.916	0	**
Cys ratio (%)	0.912	0	**
Asn ratio (%)	0.904	0	**
β-Ala ratio (%)	0.913	0	**
β-AiBA ratio (%)	-0.886	0.002	**
γ-ABA ratio (%)	0.104	0.856	-
Lys ratio (%)	0.898	0.001	**
His ratio (%)	0.086	0.882	-
Arg ratio (%)	0.149	0.76	-
Pro ratio (%)	0.907	0	**

Note: *Represents significant level; **Represents extremely significant; -represents not significant.

established retained significant effect on dependent variable of the independent variable, and selected out main independent variable affecting the dependent variable [10]. In flue-cured tobacco leaves, the content of 21 free amino acids, the ratio of each amino acid content to total free amino acid content and total free amino acids were taken as independent variables, and the burnt-mellow-sweet aroma style intensity (Y) index was taken as dependent variables. The optimal multiple linear regression equation was obtained as follows:

$$Y=-33.357+14.897X_1+4.625X_2-6.107X_3-23.336X_4-6.535X_5+10.401X_6+19.491X_7-24.807X_8+17.381X_9-12.252X_{12}-2.040X_{13}-1.156X_{14}-3.213X_{15}+38.772X_{16}-4.394X_{17}-138.220X_{18}+46.467X_{19}+0.133X_{21}+0.181X_{24}+0.433X_{25}+0.671X_{26}+0.837X_{30}+0.409X_{32}+0.199X_{33}+0.940X_{34}+0.536X_{35}+0.330X_{36}+0.605X_{37}-0.790X_{38}+5.386X_{40}+0.346X_{43}$$

Notes: Asp(X1), Glu(X2), Phe(X3), Trp(X4),Tyr, (X5), Ala(X6), Gly(X7), Ile(X8), Leu(X9), Val(X10), Thr (X11), Ser(X12), Cys (X13), Asn (X14), beta-Ala(X15), beta AiBA (X16), gamma ABA (X17), Lys (X18), His (X19), Arg (X20), Pro (X21) and total free amino acids (TFAA, X22) , Asp/TFAA (X23), Glu/TFAA (X24), Phe/TFAA (X25), Trp/TFAA (X26), Tyr/TFAA(X27), Ala/TFAA (X28), Gly/TFAA(X29), Ile/TFAA(X30), Leu/TFAA (X31), Val/TFAA (X32),Thr/TFAA (X33), Ser/TFAA (X34), Cys/TFAA (X35), Asn/TFAA (X36), beta - Ala/TFAA (X37), beta AiBA/TFAA (X38), gamma-ABA/TFAA (X39), Lys/TFAA (X40), His/TFAA (X41), Arg/TFAA (X42), Pro/TFAA (X43). The units of X1 to X22 are mg/g, X23 to X43 are %, and Y is points.

The evaluation results of free amino acid index and progressive regression equation of burnt-mellow-sweet aroma style are seen in Table 3, and the reliability evaluation of gradual regression equation established was Durbin-Waston statistic d=2.035, the determining factor R^2=0.991, analysis of variance reached a very significant level, indicating that established regression equation of burnt-mellow-sweet aroma style was more reliable. 31 indexes including Asp (X1), Glu (X2), Phe (X3), Trp (X4), Tyr (X5), Ala (X6), Gly (X7), Ile (X8), Leu (X9), Ser (X12), Cys (X13), Asn (X14), β-Ala (X15), β-AiBA (X16), γ-ABA (X17), Lys (X18), His (X19), Pro (X21) and the ratio of Glu/TFAA (X24), Phe/TFAA (X25), Trp/TFAA (X26), Ile/TFAA (X30), Val/TFAA (X32), Thr/TFAA (X33), Ser/TFAA (X34), Cys/TFAA (X35), Asn/TFAA (X36), β-Ala/TFAA (X37), β-AiBA/TFAA (X38), Lys/TFAA (X40) and Pro/TFAA (X43) jointly determined 99.1% intensity change of tobacco burnt-mellow-sweet aroma style, which was main free amino acid index affecting the intensity of burnt-mellow-sweet aroma style of flue-cured tobacco leaves.

Discussion

In this study, the content of Asp, Ala, Gly, Leu, β-AiBA, His and Trp/TFAA, Ile/TFAA, Val/TFAA, Thr/TFAA, Ser/TFAA, Cys/TFAA, Asn/TFAA, β-Ala/TFAA, Lys/TFAA, Pro/TFAA were all positively correlated with burnt-mellow-sweet aroma style of flue-cured tobacco leaves. Trp, Ile, Ser, Cys, β-Ala, Lys and β-AiBA/TFAA were significantly negatively correlated with burnt-mellow-sweet aroma style. This is not exactly consistent with the previous research conclusions on the correlation between the free amino acid index of flue-cured tobacco leaves and the intensity of fragrance type [15-17], which may be caused by the different flavor type of flue-cured tobacco leaves.

Table 3: Evaluation of free amino acid index of flue-cured tobacco leaves and the stepwise regression equation of burnt-mellow-sweet aroma style.

Sources of variation	Degrees of freedom	Sum of squares of deviation	Mean square error	F value	P value	R^2	Durbin-Waston
Repression	31	6.417	0.207	103.545	0.000	0.991	2.035
From the regression	48	0.096	0.002				
The total variation	79	6.513	0.082				

The study screened out 31 free amino acid indexes for affecting the intensity of burnt-mellow-sweet aroma style of flue-cured tobacco leaves: the content of Asp, Glu, Phe, Trp, Tyr, Ala, Gly, Ile, leu, Ser, Cys, Asn, β-Ala, β-AiBA, γ-ABA, Lys, His, Pro and proportion of Glu/TFAA, Phe/TFAA, Trp/TFA, Ile/TFAA, Val/TFAA, Thr/TFAA, Ser/TFAA, Cys/TFAA, Asn/TFAA, β-Ala/TFAA, β-AiBA/TFAA, Lys/TFAA, Pro/TFAA. Different from 4 indexes including Ala/TFAA, Ile/TFAA, the content of Gly and Tyr for discriminating the flavor types of flue-cured tobacco leaves reported by Dong [18], 25 free amino acid increased including Asp, Glu, Phe, Trp are main indexes that affects the intensity of burnt-mellow-sweet aroma style in this paper, which jointly determined 99.1% intensity change of burnt-mellow-sweet aroma style of flue-cured tobacco leaves.

Conclusion

31 free amino acid indexes were positively correlated to burnt-mellow-sweet aroma style intensity, which including the content of Asp, Glu, Phe, Trp, Tyr, Ala, Gly, Ile, Leu, Ser, Cys, Asn, β-Ala, β-AiBA, γ-ABA, Lys, His, Pro and Glu/TFAA, Phe/TFAA, Trp/TFA, Ile/TFAA, Val/TFAA, Thr/TFAA, Ser/TFAA, Cys/TFAA, Asn/TFAA, β-Ala/TFAA, β-AiBA/TFAA, Lys/TFAA, Pro/TFAA. The study provides theoretical basis for the characteristic and prediction of the intensity of burnt-mellow-sweet aroma style, which is of great significance to the formation of the technology and the use of industrial formulation for the display of burnt-mellow-sweet aroma style of flue-cured tobacco leaves.

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