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CHANGES IN COMPOSITION OF FLAVOUR PRECURSOR AMINO ACID IN LEAVES OF TEA (*Camellia sinensis*) DURING ORTHODOX BLACK TEA PROCESSING

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ABSTRACT

The content of amino acids in tea leaves of cultivars Phu Ho 11 at different stages of OTD black tea manufacture included withering, fermentation and drying was measured. Considerable changes of amino acids were observed during entire processing, among them total free amino acids were increased both in withering and fermentation stages and decreased at drying stages. The results showed that the content of some free α-amino acid contents included phenylalnine, methionine, leucine, valine and isoleucine in tea leaves were significantly increased during withering but vice versa during fermentation stage. While other amino acids such as alanine and glycine were found to be increased in both stages of processing. The zero-order, first-order and quadratic models were used to explain the α-amino acid included alanine, valine, isoleucine, leucine and phenylalanine changes kinetics during processing and it was observed that 4 models including linear, growth, compounds and quadratic could be used for explanation of the changes of flavour compounds during OTD black tea processing.

Keywords: black tea, amino acid, prediction model.

1. INTRODUCTION

Tea is one of the most popular and widely consumed beverages in the world because of its refreshing taste, attractive aroma, and potential healthy benefits. It is made from the leaves of the plant Camellia *sinensis* (L.). Generally, tea can be broadly classified according to the production method as unfermented tea (green tea), semi-fermented tea (Oolong tea), fully fermented tea (black tea) or post-fermented tea (pu-erh tea). Black tea is consumed worldwide, while green and Oolong teas are consumed mainly in Asia and North Africa. Nowadays, a lot of epidemiological and preclinical studies have demonstrated that drinking tea may reduce the risk of cancer and cardiovascular disease. Moreover, other biological functions of tea have also been reported, such as anti-inflammation, anti-oxidation, anti-allergy, and anti-obesity. These beneficial effects have been attributed to the presence of tea compounds such as polyphenols, amino acids, vitamins, carbohydrates, and purine alkaloids [1 - 3]. Theanine, c-glutamylethyl

amide or 5-N-ethyl glutamine is a non-protein amino acid that was first discovered in tea leaves. It is the main free amino acid in teas, representing as much as 50 % of the total amino acids in black tea and 1–2 % of the dry weight of green tea [3]. Free amino acids are regarded as important taste components in terms of tea quality, especially for green tea [4]. Although 26 varieties of amino acids could be found and identified in tea plants, most of them in tea leaves were theanine, glutamic acid, aspartic acid, serine, glutamine, alanine, arginine, threonine, and theanine accounted for about 60–70 % of total contents of free amino acids in spring shoots. Theanine is primarily responsible for umami (a brothy or savory) taste of green tea, similar to that of sodium glutamate [5, 6].

It has been shown that the concentration of free amino acid undergo appreciable changes during the various stages of conversion of raw material to the food products commerce. The over-all changes which occurs in the level of free amino acids during food processing included tea products suggests that they are being converted to other substances [7, 8]. Many study results show that the amino acids are being converted, at least in part, to volatile compounds likely to be important constituents of food aroma [9, 10].

Amino acids and volatile compounds play an important role in determining the character of tea [2, 6], but detailed studies of quantitative changes in this classes of compounds which occur at various stages of orthodox black tea processing included withering, rolling, fermenting and on firing have received insufficient attention especially orthodox black tea was produced from the tea clones which was cultivated at Vietnam. The present investigation presents the results of the first systematic study of progressive change in the amino acid content during each stage of manufacture.

2. MATERIALS AND METHODS

2.1. Materials

Tea leaves (*Camellia sinensis*) of cultivars PH11, representing the genetically diverse Northern Vietnam cultivars, were harvested from the Phu Tho, Vietnam were used for analyzing chemical composition. Ten kilograms of young shoots, comprising about 70 % two leaves and a bud, plus minor amounts of three leaves and a bud and loose leaf were plucked. The plucked leaf was allowed to wither under ambient conditions for 16 h and then miniature rolling–dhools. The dhool was fermented for 180 min at 30 ^{o}C. The fermentation was terminated by drying the dhool to a moisture content of about 3 % using a miniature dryer set at 120 ^{o}C inlet and 80 ^{o}C exhaust air temperature [2]. Standards of amino acid, Ala, Arg, Asn, Gly, Ile, Leu, Lys, His, Met, Phe, Ser, Thr, Glu, Trp and Tyr, were purchased from Sigma. 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) was obtained from Water Corporation, U.S.A.

2.2. Amino acid analysis

Preparation of tea infusion: Preparation of tea infusion: followed by Wang, L., et al. 0.5 g of tea powder was diluted with 30 ml of distilled water, then ultrasound-assisted extraction for 30 min. After has cooled to room temperature, it was made up to 50 ml with distilled water and filtered through a 0.45 µm filter membrane [11].

Precolumn derivatisation with OPA: The derivatisation with (AQC) reagent was carried out according to the method reported with some modifications [12] . Briefly, a 10 µl aliquot of tea infusion or standard amino acid solution was mixed with 70 µl of AccQ.FluorBorate buffer

and vortexes for 10 s. Then, 20 µl of AQC solution was added and vortexed for 10 s, and incubated at 55 ^{o}C for exactly 10 min. After, the reaction mixture was immediately used for HPLC analysis.

HPLC- Fluorescence analysis: The determination of amino acids were performed by using an HPLC system of Waters, U.S.A. The separation was completed on a Phenomenex Phenyl-Hexyl, U.S.A-C18 column (250 mm × 4.6 mm × 5 µm). The temperature of column oven was set at 37 ^{o}C. The mobile phase consisted of acetonitrile/water (60/40, B) and buffer Waters AccQ.Tag (A). The flow rate was 1.0 ml m^{-1}. Optimal excitation (λex) and emission (λem) wavelengths for each OPA-amino acid derivative for fluorometric detection: 250-nm to 395-nm.

2.5. Statistical analysis

Statistical comparisons of the mean values for each experiment were performed by one-way analysis of variance (ANOVA) by using SPSS 11.5 for Windows software. Significance was declared at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Changes in amino acid profile during processing

Table 1. Changing of amino acids content during processing of PH11 cultivar.

Flavours precursors	Fresh leaf	Withered leaf			Rolled	Fermented leaf			Dried
		At 6h	At 12 h	At 16 h		At 60 m	At 120 m	At 180 m	
	mg/100g dry basis								
Aspartic acid	48.01	240.58	263.87	260.84	175.94	181.31	210.51	544.24	154.18
Alanine	15.34	33.25	36.76	29.07	17.16	21.51	25.42	38.11	20.12
Methionine	6.00	12.24	18.10	21.07	17.15	16.03	15.01	14.11	12.23
Histidine	36.25	42.32	45.71	51.34	72.0	82.03	91.45	98.11	42.24
Arginine	90.15	121.46	139.66	141.90	96.39	97.36	162.72	216.61	133.72
Serine	276.92	470.24	417.56	877.17	448.72	464.04	628.2	1275.94	836.43
Glutamic acid	332.19	297.52	326.58	358.2	119.74	93.64	221.11	420.32	135.29
Glycine	146.29	179.87	195.13	206.33	79.64	106.69	163.47	366.45	61.05
Threonine	426.67	510.67	550.19	590.23	380.22	419.11	458.09	493.11	109.03
Tyrosine	120.08	136.55	118.93	85.03	66.05	74.75	94.16	138.38	84.75
Theanine	1092.94	1643.61	1602.61	1308.11	1395.32	1484.16	1335.24	988.96	811.84
Lysine	236.96	244.70	232.04	210.87	168.34	159.02	171.76	208.82	155.91
Valine	90.60	202.5	331.23	433.12	394.99	361.21	351.14	331.35	319.42
Isoleucine	15.83	46.24	70.19	88.31	84.61	87.24	79.66	61.88	54.96
Leucine	35.72	56.22	142.63	152.01	150.06	145.51	141.48	128.91	109.52
Phenylalnine	35.9	61.91	146.33	158.06	139.99	128.84	118.35	113.12	82.41
Total amino acids	3005.85	4299.88	4637.52	4971.66	3806.32	3922.45	4267.77	5438.42	3123.1

The study revealed that significant amino acids composition changes took place during OTD black tea processing from cultivars PH11. The changes of amino acids content during OTD black tea processing which was demonstrated as flavour precursors was shown in Table 1. The results showed that the contents of some free α-amino acid included phenylalnine, methionine, leucine, valine and isoleucine in tea leaves were significantly increased during withering (35.9 - 158.06 mg/100g; 6.00 - 21.07 mg/100g; 35.72 - 152.01 mg/100g; 90.60 - 433.12 mg/100g and 15.83- 88.31 mg/100g, respectively) but vice versa during fermentation stage (139.99 -113.12 mg/100g; 17.15 - 14.11 mg/100g; 150.06 - 128.91 mg/100g; 394.99 - 331.35 mg/100g and 84.61 - 61.88 mg/100g, respectively). Other α-amino acid contents such as alanine and glycine were found to be increased in both stages of processing i.e. withering (15.34 - 29.07 mg/100g; 146.29 - 206.33 mg/100g, respectively) and fermentation (17.16 - 38.11 mg/100g; 79.64 - 366.45 mg/100g, respectively). In the entire process, the content of free amino acids in fermented tea leave was higher than in fresh tea leaves. In previous studies it was mentioned that apart from protein breakdown, sugar is also coverted into amino acid during processing and an increase in total free amino acid during both the withering and fermation stage of tea manufacture was confirmed [13].

In some previous studies, it has showed that the α-amino acids present in tea leaves material will undergo Strecker degradation to form corresponding aldehydes in the presence of, and only in presence of, oxidizing tea flavanols. In principle, all free amino acids should have their corresponding Strecker aldehydes. However, only amino acids such as glycine, alanine, valine, leucine, phenylalanine, isoleucine and methionine have their Strecker aldehydes and their corresponding Strecker aldehydes are formaldehyde, acetaldehyde, isobutyraldehyde, isovaleraldehyde, phenylalacetaldehyde, 2-methylbutanal and methional, respectively. One reason is that non-volatile products are generated instead of volatile aldehydes. The other possibility is some Strecker aldehydes are so unstable that they readily decompose into other volatiles by cyclization, coupling, or dehydration [14, 15]. The results showed that α-amino acids had increasing trend in withering stages while it was observed inversely i.e. decreasing trend during fermentation stage frying stages.

3.2. Prediction models for amino acids changes during withering and fermentation

For the mathematical predicting of flavor precursors i.e α-amino acids changes during OTD tea withering stage and fermation stage, quadratic, zero-order and first-order models were used. The model, that was chosen when it had the highest value of adjuted R^2 and the lowest value of p ($p \leq 0.05$). It was observed that at withering stage, two amino acids (alanine and tyrosine) were fitted to the quadratic model; on the other hand, the values of phenyalnine and leucine followed a compound model, while the changes of isoleucine, valine content during withering stage were fitted to the linear model.

At fermentation stage, it was found that alanine, phenyalnine, tyrosine and valine were fitted to growth model, and isoleucine was fitted to the quadratic model. The estimated prediction parameters of these models and the statistical values of coefficients of determination adjusted R^2 as well as significant values are represented in Table 2. The presented results were in agreement with the studies published in the literature and several previous studies [13, 16 – 18].

Table 2. Model summary, ANOVA and Coefficients of prediction model for amino acids changes.

Flavour precursor	Model	Equation	Adjusted R^2	p (ANOVA)	p (Coefficient)	
		Withering stage				
Alanine	Quadratic	$Y = 15.220 + 4.383*t - 0.219*t^2$	0.995	0.041	T	0.029
					T^2	0.035
					C	0.027
Phenyalnine	Compound	$Y = 36.429 *(1.105^t)$	0.942	0.019	T	0.000
					C	0.021
Leucine	Compound	$Y = 34.974*(1.104^t)$	0.926	0.025	T	0.000
					C	0.027
Tyrosine	Quadratic	$Y = 119.890 + 5.899*t - 0.504*t^2$	0.998	0.028	T	0.034
					T^2	0.024
					C	0.005
Isoleucine	Linear	$Y =17.104 + 4.475*t$	0.996	0.001	T	0.001
					C	0.009
Valine	Linear	$Y = 83.298 + 21.302*t$	0.994	0.002	T	0.002
					C	0.014
		Fermentaion stage				
Alanine	Growth	$Y = e^{(2.813+0.004*t)}$	0.945	0.018	T	0.018
					C	0.001
Phenyalnine	Growth	$Y = e^{(4.934-0.001*t)}$	0.975	0.008	T	0.008
					C	0.000
Leucine	Linear	$Y = 151.161 - 0.112*t$	0.878	0.042	T	0.042
					C	0.000
Tyrosine	Growth	$Y = e^{(4.127+0.004*t)}$	0.919	0.027	T	0.027
					C	0.000
Isoleucine	Quadratic	$Y =84.611+ 0.129*t - 0.001*t^2$	1.000	0.000	T	0.000
					T^2	0.000
					C	0.000
Valine	Growth	$Y = e^{(5.966-0.001*t)}$	0.939	0.021	T	0.021
					C	0.000

** t –time; C, T, T^2- Significantly coefficient value of Constant in Equation, consequently.*

4. CONCLUSION

Considerable changes of amino acids in tea leaves of cultivars PH11were observed in all stages but in each stage, total free amino acids were increased in withering stages from 3005.85 mg/100g to 4971.66 mg/100g after 16 hours withered and keep continuously during fermentation stage with the content was found at 5438.42 mg/100g. The total content of amino acids significantly decreased after drying stage about 40 % in comparing with the content of fermented leaves (from 5438.42 mg/100g in fermented leaves to 3123.1 mg/100 g). The contents of some free α-amino acid which was defined as flavour precursor such as phenylalnine, methionine, leucine, valine and isoleucine in tea leaves were significantly increased during withering whereas they were found to have

decreasing trend during fermentation, while other α-amino acid contents including alanine and glycine were found to be increased in both stage of processing i.e withering and fermentation.

The zero-order, first-order and quadratic models were used to explain the flavour precursors changes kinetics during processing and it was observed that the 4 models (linear, growth, compounds and quadratic)could be used to explain the changes of flavour compounds during OTD black tea manufature. To the best of our knowledge, this is the first report using multivariable analysis coupled with intrusment method to modeling the changes of amino acid during OTD black tea manufacture.

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TÓM TẮT

SỰ BIẾN ĐỔI THÀNH PHẦN TIỀN CHẤT TẠO MÙI AMINO ACID CỦA LÁ CHÈ *(CAMELLIA SINENSIS)* TRONG QUÁ TRÌNH CHẾ BIẾN SẢN XUẤT CHÈ ĐEN OTD

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Thành phần acid amin trong lá chè thuộc giống chè Phú Hộ 11 được phân tích ở các công đoạn của chế biến chè đen OTD bao gồm công đoạn héo, lên men và sấy. Trong quá trình chế biến, hàm lượng axit amin có sự thay đổi đáng kể trong đó hàm lượng axit amin tổng số có xu hướng tăng ở cả hai giai đoạn chế biến gồm héo và lên men và hàm lượng giảm mạnh sau công đoạn sấy chè. Kết quả nghiên cứu cũng cho thấy hàm lượng một số α-axit amin được xem là tiền chất tạo mùi như phenylalnine, methionine, leucine, valine và isoleucine tăng nhanh trong công đoạn héo nhưng lại có xu hướng giảm trong công đoạn lên men, trong khi đó hàm lượng các α- axit amin khác như alanine và glycine có xu hướng tăng ở cả hai công đoạn. Mô hình hồi quy zero-order, first-order và quadratic được sử dụng để giải thích sự biến đổi hàm lượng của các α-amino acid trong bao gồm alanine, valine, isoleucine, leucine và phenylalanine trong quá trình chế biến chè đen ở hai giai đoạn héo và lên men. Kết quả xử lí cho thấy các mô hình dạng linear, growth, compound và quadratic có thể sử dụng để mô tả sự biến đổi của các α-amino acid trong quá trình chế biến chè đen OTD.

Từ khóa: chè đen, acid amin.