Isolation of Tea Brown Pigment from Pu'er Tea and Its Balance of Human Metabolic Function

Shan Zhiguo[1], Zhang Chunhua[1,*], Qiang Jiye[1], Man Hongping[2]

[1]Pu'er University, Pu'er 665000, China

[2]Pu'er Comprehensive Technical Testing Center, Pu'er 665000, China

Email: 2017117943@qq.com

Keywords: Pu'er Tea; Theophyllin; Separation; Human Metabolism

Abstract: Pu 'er tea is a kind of post-fermentation tea with various health functions, of which polyphenol and theabrownin are important functional components. Pu 'er tea theabrownin is a kind of polyphenol oxidation product with very complex structure and belongs to natural pigment. Analysis bags with different molecular entrapments can effectively separate theabrownins. AFM showed that the morphologies of theabrownin particles with different molecular weights were not uniform, and the single molecule was in island state or granular aggregate structure. When the particles gather more, they are linearly linked and have more branches or form a network structure.The macroporous resin was used to separate the tea brown pigment into two components with different polarities. The DEAE-52 anion exchange resin was used to separate the components with higher polarity and low phenolic content according to the difference of ionic strength. Several different properties of the tea brown fraction. Different components of the tea brown pigment have a certain difference in total sugar, total phenol and protein content. The four major components of TBsP1 isolated have similar composition and different contents, which provide reference for studying the composition, structure and function of the tea brown pigments.

1. Introduction

Pu 'er tea is a kind of post-fermentation tea with various health functions, of which polyphenol and theabrownin are important functional components. Pu 'er tea theabrownin is a kind of polyphenol oxidation product with very complex structure and belongs to natural pigment. Analysis bags with different molecular entrapments can effectively separate theabrownins. AFM showed that the morphologies of theabrownin particles with different molecular weights were not uniform, and the single molecule was in island state or granular aggregate structure. Pu 'er tea with linear links and many branches or reticular structures is unique in China when the particles gather more, and is prepared by pile fermentation of Yunnan big-leaf sun-dried green tea [1]. Pile fermentation is the key to the formation of Pu 'er tea quality. Its essence is that the chemical substances in the tea undergo a series of complex chemical reactions such as transformation, isomerization, degradation, polymerization, coupling and so on under the combined action of enzymatic and moist heat with the participation of microorganisms to form the unique quality of Pu 'er tea [2]. During pile fermentation, tea polyphenols are oxidized into theaflavins and thearubigins under the action of microorganisms and damp heat. Theaflavin and thearubigins undergo further oxidative polymerization and combine with compounds such as polysaccharide, protein and lipid to form theabrownin [3].Tea brown pigment is soluble in water and insoluble in ethyl acetate and n-butanol. It is a very complex compound. It has anti-cancer, weight loss, lipid-lowering, anti-fatigue and other active functions [4]. At present, the research on theaflavins is the most in-depth, and the research on thearubigin has made some progress [5]. However, due to its complex composition and structure, the tea brown pigment is difficult to separate and purify, and its research progress is slow.

Pu 'er tea can regulate the metabolism of the body, mainly including three aspects [6]. First, theabrownin and other active ingredients with surfactant have strong cholesterol binding ability with fats, especially saturated fatty acids. Inhibit its absorption and increase excretion, thus

  DOI: 10.25236/medsbe.2019.008

preventing the human body from absorbing food with high energy [7]. Second, Pu 'er tea water soluble substances can inhibit key enzymes in the process of fat metabolism, thus reducing liver, muscle and free adipose tissue. The third is to regulate glucose metabolism in blood by regulating lipid metabolism [8].At present, metabolomics, as an emerging discipline, has become a research for a variety of diseases and drug toxicity. The existing metabolomics research on tea is mainly concentrated with black tea and green tea. Because the chemical characteristics of Pu'er tea are significantly different from those of black tea green tea [9]. Therefore, it is urgent to deepen our metabolic process in the body of this unique fully fermented tea, and the objective understanding of the corresponding role of the human body, so that it can better serve the mechanism of drug screening and health regulation [10].

2. Analysis on the Structure and Change of Tea Brown in Puer Tea

2.1. Structural analysis of theophyllin

Theabrownin refers to a kind of brown pigment which is soluble in water but insoluble in ethyl acetate, n-butanol and ethanol. It is also a very complex compound. In order to investigate the composition of theabrownin in Pu 'er tea, Li Lianxi and others used ultrafiltration membrane filtration combined with component detection, and concluded that the tea brownin in Pu 'er tea not only contains oxidation polymerization products of polyphenols, but also contains amino acids, nitrogen-containing substances, sugars, caffeine and other combinations. After theabrownin is formed, some of it can combine with protein and precipitate on the leaf bottom, forming the color of the leaf bottom. Based on the formation, chemical properties and structural characteristics of humic acid, Li Baocai et al. proposed that the chemical structure and functional groups of Pu'er tea brown pigment and humic acid have some similarities. That is, they all contain a large amount of phenolic groups, carboxyl groups, and sulfhydryl groups, and their basic cores are all aryl groups. Although the connection method is different, there is a structure-activity relationship, but the tea pigment and humic acid should be pharmacologically a phenolic group, a carboxyl group, a thiol group, and the like. From this, it can be inferred that humic acid has some similarities with lycopene in pharmacology.

2.2. Changes of theanin during storage and its relationship with quality

During the storage of Pu 'er tea, the content of theabrownin has been increasing, which plays a very important role in the formation of Pu 'er tea quality. Natural storage and aging for a certain period of time are necessary for the formation of Pu 'er tea quality, but the longer the storage time, the better. According to Zhou Hongjie's research, the ratio of theaflavin, thearubigins and theabrownin in the finished Pu 'er tea increased by 54.7%, 12.6% and 25.5% respectively after storage for half a year, forming the orange and bright soup color of Pu 'er tea. With the prolonging of storage time, theaflavin and thearubigins will be degraded by auto-oxidation or gradually generated into theabrownin by interaction with protein and amino acid.Pu'er tea, theaflavins and theaflavins, which were stored for one year, decreased significantly, while the tea brown pigment increased significantly, turning the soup color dark. This is also the main reason why the taste of tea is light and the aroma is aged and even mildewed. However, in the process of aging and drying, the water-soluble pigment and the water-insoluble pigment remaining in the leaf bottom change, and its relationship with the quality of Pu'er tea remains to be further studied.

3. Physiological Effects of Tea Brown in Puer Tea

3.1. Antioxidant function

Oxidation is the greatest threat to skin aging. Unhealthy diet, sun exposure and environmental pollution can lead to the oxidation of skin free radicals, resulting in dim skin color, water shortage and other symptoms. Ni Dejiang and others used Pu 'er tea as raw material to extract its water extract with ethyl acetate and n-butanol. The antioxidant capacity of theabrownin extract from Pu

'er tea was studied. The results show that the extract has certain antioxidant activity, and the antioxidant activity is higher than Pu 'er tea water extract.Dong Wenming et al studied the antioxidant capacity of the tea brown pigment extract under different processing and storage conditions of Pu'er tea. The results showed that the fermentation time, process, storage period and the concentration and pH value of the tea brown pigment extract could affect its removal. The effect of free radicals. With the increase of concentration, the free radical scavenging ability of the tea brown pigment extract increased gradually, and the fourth turn-up sample removal rate was the highest during fermentation. Chen Yuqiong et al studied the antioxidant properties of different components of Pu'er tea, and obtained the ability of tea brown pigment to scavenge free radicals.

3.2. The function of reducing fat and weight

Obesity is a state of overweight caused by excessive accumulation of fat in the body, which often causes various complications and accelerates aging and death. Huang et al. used Pu 'er tea, green tea and black tea extracts to activate AMP-dependent protein kinase in high-fat rats to inhibit the activities of hyperlipidemia and fatty acid synthase. The results showed that Pu 'er tea had better inhibitory effect than other teas. Chen Ting and others have verified the lipid-lowering and weight-reducing effects of theabrownin through animal experiments. The results show that theabrownin of Pu 'er tea can significantly reduce the levels of total cholesterol, triglyceride and low density lipoprotein in high-fat rats and increase the level of high density lipoprotein. Reduce liver deposition and prevent the formation of fatty liver.Like Chen Ting's experimental conclusion, Peng, Gong, etc. used Pu'er tea as raw material to extract the tea brown pigment. It was applied to animal experiments and tested for cholesterol and bile acid content in rat feces. It was found that tea brown pigment not only promotes the conversion of cholesterol, but also promotes the excretion of cholesterol in rats. Tea brown pigment has proved to be a natural lipid-lowering weight loss functional component through animal experiments, and has the potential to deeply research and develop end products.

4. Separation of Camellin from Puer Tea

4.1. Separated materials and methods

Prepare cation exchange resins 001X7, D72, D61, D151 provided by Nankai university chemical plant, anion exchange resins D280, D201, D301T and macroporous adsorption resins ab-8, D4006, nka-9, d352deae-52 produced by Beijing huamaike biotechnology co., ltd. besides, after the collection of materials and instruments such as 1-phenyl-3-methyl-5-pyrazolone (PMP) of Shanghai siyu chemical industry, the preparation of theabrownin will begin. After Pu 'er tea is pulverized, it is extracted with 95% ethanol for 8 h each time and three times. The extract is substantially colorless and the alcohol soluble material is removed. The remaining tea powder was extracted with hot water at 60 ° C for three times at a water addition ratio of 1:20 (w/v), and the three extracted tea soups were combined. The filter paper was filtered off to remove suspended particles, and concentrated by steaming at 60 ° C until the volume of the concentrate was 1/10 before concentration, and concentrated in a ratio of 1:1 (v / v) with ethyl acetate, dichloromethane and n-butanol. The liquid is extracted. Each organic solvent was extracted three times to obtain a residual aqueous layer, and the organic solvent was removed by rotary evaporation at 60 ° C, and lyophilized to obtain a tea brown pigment powder, which was reconstituted and stored at 4 ° C until use.

4.2. Separation results and analysis

The static adsorption curve of theabrownin of ab-8 resin can be seen from figure 1, when ab-8 resin adsorbs for 30min, the adsorption rate of total phenol reaches 62.95%, and the adsorption rate of total sugar reaches 19.34%. After adsorption for 1 hour, the adsorption rate of total phenol reached 70.80%, and the adsorption rate of total sugar reached 30.97%, then the adsorption rate changed little. The effect of theabrownin total phenol concentration on ab-8 resin adsorption can be

seen from figure 2, when the total phenol concentration in theabrownin is less than 10mg/mL, the adsorption amount of ab-8 resin is proportional to the concentration. When the total phenol concentration exceeds 10mg/mL, the adsorption amount of AB-8 resin to the total phenol in theabrownin does not increase much, and the adsorption amount of AB-8 resin to the total phenol in theabrownin is about 55 mg/g. PH has certain influence on the adsorption performance of AB-8 resin. The pH of the crude tea brown pigment solution is about 6.2-6.8. The pH change affects the dissociation of the phenolic hydroxyl group in the tea brown pigment. The AB-8 resin has the highest adsorption amount of total phenol at pH 4.0, AB- The adsorption rate of the resin to the total sugar decreased as the pH increased. Too low or too high a pH will affect the formation of hydrogen bonds, and pH 4.0 is selected as the optimum adsorption pH of the AB-8 resin.

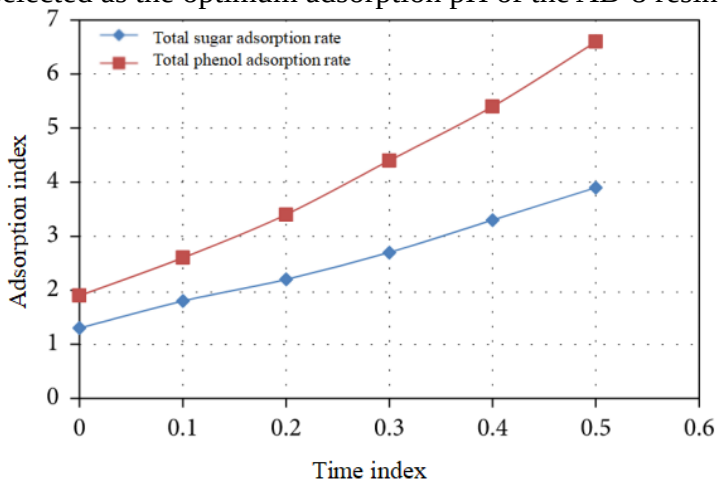

Fig.1. Static adsorption curve of AB-8 resin

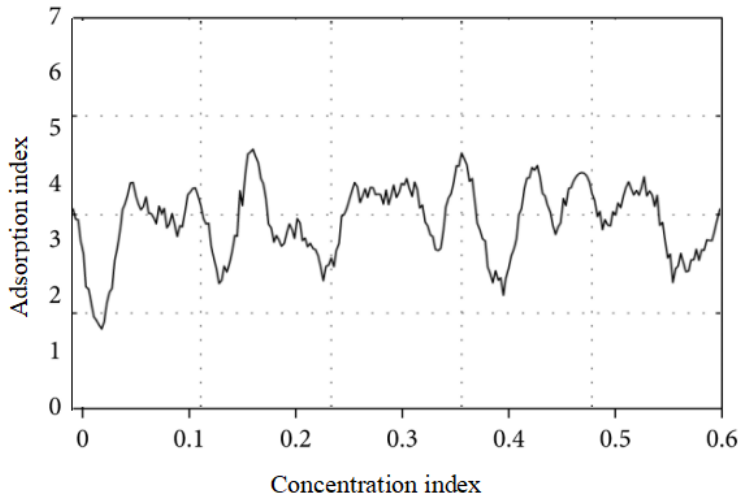

Fig.2. Effect of total phenol concentration of theaflavins on adsorption of AB-8 resin

5. Study on the Balance of Metabolic Function of Pu'er Tea

5.1. Metabolomic study on the effect of Pu'er tea on human metabolism

The establishment of a standardized metabolome fingerprint spectrum acquisition method system specifically includes studying the extraction and separation methods of metabolite components in biological samples and their standardization. Emphasis is placed on the study of convenient and fast treatment technologies such as high-speed centrifugation and ultrafiltration. To study the optimization method of chromatographic separation conditions, to study and establish a method for obtaining metabolite fingerprint spectrum combining separation analysis, non-separation analysis and computational analysis, and to analyze and identify the components of characteristic metabolites in fingerprint spectrum. This paper studies the computer automatic identification

algorithm of the peak position in the fingerprint spectrum, and accurately identifies and automatically matches each fingerprint peak of the fingerprint spectrum under the condition that the peak position changes randomly.In addition, the characteristic metabolic model of drinking Pu'er tea and the comparison of several teas. Metabolomics studies were conducted using the normal population as experimental subjects, using computational techniques such as instrumental analysis and bioinformatics and data analysis. The characteristic metabolic patterns of drinking Pu'er tea were summarized and found to be differential metabolic markers, and their effects were demonstrated by combining chemical composition and metabolomics.

5.2. Metabonomics method for the effect of Pu'er tea on human metabolism

The first is the establishment of analytical methods. As a part of system biology, metabonomics research needs good sensitivity, quantitative data and stability of analytical methods. At present, the main applied methods are NMR, LC/MS and LC/MS/MS,CE/MS and GC/ms. Nuclear magnetic resonance (NMR) and mass spectrometry (MS) can rapidly analyze multiple substances in complex samples from a variety of analytical instruments and methods, and are good analytical platforms for metabonomics research. However, the application of mass spectrometry in metabonomics reflects the characteristics of organisms more accurately than nuclear magnetic resonance. The development of chromatography technology is the cornerstone of modern analytical science. It can separate complex compounds in a short time.The combination of chromatography and mass spectrometry makes it possible to simultaneously quantify and qualitatively analyze complex metabolites. In the field of metabolomics research, due to the unique advantages of chromatography and mass spectrometry, its application is increasingly widespread. Through a series of experimental conditions optimization and methodological investigation, we established a UPLC/Q-TOF-MS method for the study of human metabolism in Pu'er tea. The metabolic detection experimental method is shown in Table 1.

Table 1 Mobile phase elution gradient in metabolic detection

Time(min)	Flow rate(min)	a (%)	b (%)	curve
0	0.5	98	1	0
0.5	0.5	98	1	5
5	0.5	79	21	5
9	0.5	5	96	5
10	0.5	5	96	5
13	0.5	98	1	1

6. Conclusion

China is the hometown of Pu 'er tea. In recent years, many experts and scholars in China have reported on Pu 'er tea and confirmed that Pu 'er tea has outstanding biological activities in regulating blood lipid, antioxidant, anti-tumor, immune regulation, hypoglycemic and other aspects. Through the research on the separation method of theabrownin, several components of theabrownin were obtained. The contents of total sugar, total cheese and protein in each component were determined, and the monosaccharide composition of the main separated components was analyzed. The antioxidant activity of each component was compared, which provided theoretical basis for the research and utilization of theabrownin.However, according to the research status, there are still some problems in the exploration of tea brown pigment in Pu'er tea. The structure of tea brown pigment is complex, and its composition is still not clear so far. It is very difficult to separate and purify. The biological activity exhibited by Pu'er tea pigment is a composite effect of several functional components, and the function of the tea brown pigment has not yet been discussed. There is currently no practical way to detect the brown pigment, which is the place to continue to explore.

Acknowledgement

In this paper, the research was sponsored by the Pu'er College High-level Talents Research Initiation Project (K2015032); Pu'er Tea Experimental and Practical Training Base and Processing Technology Innovation Service Center (A03047-16); Pu'er College Key Project (K2018012); Yunnan Provincial Education Department Project (K2017058); Yunnan Provincial Education Department Science Research Fund Project (2018JS513).

The study was supported by “Science and Technology Project of China Railway Corporation, China (Grant No. 1341324011)”.

References

[1] Elmastas M, Celik S M, Genc N. Antioxidant activity of an anatolian herbal tea-origanum minutiflorum: Isolation and characterization of its secondary metabolites. International Journal of Food Properties, 2018, 21(1):374-384.

[2] Zhang J. Puer tea: Ancient caravans and urban chic. Asia Pacific Journal of Anthropology, 2014, 17(3-4):361-363.

[3] Shuenn-Der. Taiwan and the Globalization of Puer Tea: The Role of the Taste of Aging. 2016(5):310-320.

[4] Suttekiti M. Isolation of Vitamin C from Vegetables and the Relations between Vitamin C and Ascorbic Acid. Bulletin of the Agricultural Chemical Society of Japan, 2014, 10(10-12):184-192.

[5] Chen Z M, Lin Z. Tea and human health: biomedical functions of tea active components and current issues. Journal of Zhejiang University Science B, 2015, 16(2):87-102.

[6] Pfeil B E, Schoefs B, Spetea C. Function and evolution of channels and transporters in photosynthetic membranes. Cellular and Molecular Life Sciences, 2014, 71(6):979-998.

[7] Cai X, Huang Q, Wang S. Isolation of a novel lutein–protein complex from Chlorella vulgaris and its functional properties. Food Funct. 2015, 6(6):1893-1899.

[8] Isolation and identification of a gene encoding 4-hydroxyphenylpyruvate dioxygenase from the red-brown pigment-producing bacteriumAlteromonas stellipolarisLMG 21856. Folia Microbiologica, 2015, 60(4):309-316.

[9] Zou Y, Qi G, Chen S. A simple method based on image processing to estimate primary pigment levels of Sichuan Dark Tea during post-fermentation. European Food Research and Technology, 2014, 239(2):357-363.

[10] Caffeine is responsible for the bloodglucose-lowering effects of green tea and Puer tea extractsin BALB/c mice. Chinese Journal of Natural Medicines, 2015, 13(8):595-601.