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Review Ariticle

The Research Progress of Main Chemical Constituents and Functional Activity in Purple Tea

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Abstract: Purple tea is made from the traditional processing of green tea, black tea and dark tea, which will maintain the purple characteristic of the tea soup during the processing. It has been widely concerned and studied due to its high anthocyanin content, unique color and biological activities such as scavenging free radicals, anti-oxidation, anti-cancer, anti-inflammatory, anti-bacterial, protecting eyesight and relieving visual fatigue. In addition, purple tea also contains active ingredients such as tea polysaccharides and tea polyphenols. At present, although purple tea is widely used in skin care, food, medicine and other fields, the research on purple tea is still in the exploratory stage, and its unique economic value has not been fully utilized. In order to improve processing technology, develop high-quality products to meet market demand, and improve the stability of anthocyanins in tea extracts, this paper is about of the varieties of purple tea, the effects of different processing methods on the quality, the analysis of color change mechanism of purple tea leaf, the chemical composition and its physiologically active substances, metabolic pathways and gene expression. And the key research directions and new product development of purple tea are prospected, which provides a further reference for related research on purple tea.

Keywords: Purple Tea, Processing, Composition, Functional Activity, Anthocyanin

1. Introduction

Purple tea is made from the traditional processing of green tea, black tea and dark tea, which will maintain the purple characteristic of the tea soup during the processing, such as Zijuan tea, Taixiangzi tea (C. sinensis cv.) and so on. In recent years, purple tea has been widely concerned about its unique economic value due to high content of anthocyanins and special functional activities [1-5]. Compared with other ordinary teas such as Fuding Dabai and high-quality teas in most countries, purple tea has a special color and biological activity. In addition, its distinctive aroma and taste are also popular among consumers. Purple tea has been used in skin care, food and medicine fields [6]. Obviously, purple tea has significant research value, but the research on purple tea is still in the exploratory stage [7]. This paper summarizes the varieties of purple tea, the influence of different processing methods on the quality of purple tea, analysis of color change mechanism of purple tea leaf, effects of chemical components and bioactive products of purple tea. The pivotal research direction and new product development are prospected to provide reference for the further research of purple tea.

2. Purple Tea Variety

The breeding of purple tea in China began in the mid-1980s, including Zijuan tea [8, 9], Chinese red bud Buddha tea [10], Taixiangzi tea and Ziyan tea [11, 12], and anthocyanin content of purple tea is commonly higher compared with green tea. Japanese scientists discovered anthocyanin-rich tea varieties in their country in the early 20th century, such as Benibana-cha [13] and "Sunrouge" [14]. Benibana-cha mainly contains two kinds of anthocyanins which are delphinidin and cyanidin 3-O-β-d-galactosides while "Sunrouge" contains four which were identified as delphinidin-3-O-β-D-(6-(E)-p-coumaroyl)galactopyranoside, delphinidin-3-O--D-(6-(E)-p-coumaroyl)glucopyranoside (3), cyanidin-3-β-D-(6-(E)-p-coumaroyl)galactopyranoside, and cyanidin-3-O-β-D-(6-(E)-p-coumaroyl) glucopyranoside. Zijuan tea is a kind of puer tea, which is unique to Yunnan [8], and the anthocyanin content in the new buds is 100 times that of the Japanese high-anthocyanin tea variety "Zhengden 03-1384". Kenyan scientists have reported that some new varieties have a higher content of tea polyphenols than green tea, and retain their biochemical composition, antioxidant capacity and biological activity, including TRFK K-Purple, TRFK KS1, TRFK 306/1 [15-16]. Indian scientists have reported that some purple tea varieties grown in the Kangra Valley [17] have higher polyphenol content compared with green tea, higher theaflavins content compared with black tea, and stronger antioxidant properties. In addition, the volatile flavor of purple tea is more stable.

In summary, it can be seen that the breeding of purple tea has existed at home and abroad, which provides a reference to breed new types of purple tea, and help developing some tea products rich in anthocyanins [18].

3. Effects of Different Processing Methods on the Quality of Purple Tea

Purple tea has stronger antioxidant capacity than traditional varieties due to its higher anthocyanin content. The difference in processing technology will reduce the antioxidant activity of anthocyanins to varying degrees, change the color of purple tea, and affect the activity of functional substances. Tea processing methods are diverse in China, so it is very important to study the effects of different processing methods on the components of purple tea.

Researchers in Kenya [19] compared the components of purple tea made by processing technology of green tea and black tea, which showed that the loss of anthocyanin caused by black tea process was high. In addition, a (EGCG +ECG) /EGC index model (Yuan (1962) showed that the quality index number [(EGCG +ECG) /EGC] could be used as the grading standard of green tea: the higher the index, the better the quality of green tea. Moreover, green tea made from purple tea was superior to other green tea varieties in flavor. Meanwhile, Indian researchers [18] used green tea process and black tea process to make purple tea. The results showed that there was no anthocyanin in green tea process while little in black tea pocess. The reason might be that the convection microwave oven was used to kill the anthocyanin, which led to excessive decomposition of anthocyanin. Therefore, a more detailed study is needed to determine which sterilization methods (roller fixation, hot air fixation, infrared fixation, microwave fixation, etc.) can maintain the nutrition and flavor of purple tea to the greatest extent.

Shi studied the influence of different processing techniques on the quality of azalea tea [20] and reported that the higher the degree of fermentation, the brighter the color. The levels of catechins, tea polyphenols and anthocyanins will decrease as the ethanol concentration increases. Therefore, not only the color and taste should be considered, but also the loss of tea polyphenols and anthocyanins should be reduced during the processing of purple tea.

Domestic researchers [13, 19, 21] processed purple tea by making green tea, black tea and oolong tea. And the green tea was divided into roasted green tea, steamed green tea and suntan green tea. The results showed that the anthocyanin content of unfermented green tea was significantly higher than that of semi-fermented oolong tea and fully fermented black tea. The content of anthocyanin in roasted green tea was the highest. In addition, the aroma components of roasted green tea are more complex and a variety of unique aroma components have been identified [22, 23].

Purple tea has great potential after long-term storage, and its value will become higher and higher due to its softer aroma and more transparent wine color. Different processing methods will result in the loss of anthocyanins in purple tea. To some extent, it will weaken the flavor and color of purple tea, and reduce the nutritional content, thereby affecting its commercial value. Therefore, the processing method should be considered when processing purple tea, and the original quality of purple tea should be maintained as much as possible. ain the original quality of purple tea.

4. Analysis of Color Change of Purple Tea Leaf

The color of purple tea leaves changes from purple to green in the development stage, which is mainly positively correlated with the total amount of anthocyanin/chlorophyll [24].

Shen et al. [72] used Ultra high Performance liquid chromatography-Quadrupole time-flight mass spectrometer (UPLC-QTOF-MS) to analyze the metabolites of Zixin leaves in all purple, medium purple and all green phases. And the results showed that the change of leaf color was mainly caused by the decrease of flavonoid/anthocyanin content. This is helpful to study the color change mechanism of purple tea leaf. Kumari et al. [5] studied the metabolic process of color transformation of purple tea leaf at the green and young stages (JG), light purple stage (LP) and deep purple stage (DP). Studies have shown that the direct or indirect interaction of proteins, photosynthesis, transcription factors and anthocyanin biosynthesis involved in primary and prophase metabolism leads to the color change of purple tea leaves. It provides a basis for the future research on the color discoloration mechanism of purple tea leaf. Chen et al. [25] studied the effect of different light treatments on the color of berberis berberis leaves. The results showed that the color of leaves of berberis was mostly deep purple under light treatment, while the color of leaves was light purple or light green under shade treatment. The results showed that the shading treatment could increase the chlorophyll content, while decrease the activity of phenylalanine ammonia-lyase. This causes the color of the leaves to change to green. This provides a theoretical basis for us to keep sufficient light when planting amur berberis. All the above studies have confirmed that the color change of purple tea leaf is related to the content of anthocyanin and leaf green.

5. Analysis of Anthocyanin, the Main Component of Purple Tea

The rich anthocyanin content of purple tea highlights its value. Anthocyanin is a representative component, and the study of its metabolism and accumulation pathway and component analysis are of substantial significance to the development of the value of purple tea. Therefore, many scholars at home and abroad have conducted in-depth studies on this issue. Some scholars have also analyzed other components of purple tea to explore the interaction between each component and its characteristics and flavor.

5.1. Analysis of Anthocyanin Structure and Component

Anthocyanins are mainly found in petals, fruits, rhizomes and other plant parts. It is a water-soluble pigment [26], which is one of the main pigments that make up petals and fruits. Its structure is shown in Figure 1. Different anthocyanins have different substituents at the R1 and R2 positions of the anthocyanins and belong to the flavonoids [27].

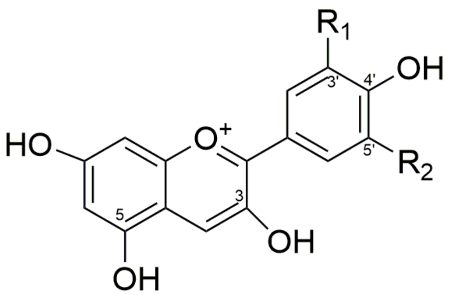

Figure 1. *The structure of anthocyanin.*

Anthocyanin content in Zijuan tea is about 0.5573 mg/g, which were determined by tea Research Institute of Chinese Academy of Agricultural Sciences. In addition, anthocyanin in Zijuan tea is composed of geranium - 3, 5 - two glycosidase, cornflower element - 3 - galactose glucoside, cornflower – 3 - O - glucoside, delphinium, cornflower, geranium, peony and mallow flowers. And the anthocyanins - 3 - galactose glucoside accounts for the most. And the content distribution of anthocyanin in different mature leaves of Zijuan tea was determined, which proved that the content of anthocyanin in the second leaf (the second leaf of bud) was the highest. Sichuan Agricultural University [11] analyzed the anthocyanin components of Ziyan, and measured that the anthocyanin content was about 0.8815 mg/g, which was significantly higher than the current purple tea variety. However, only delphinidin, cornflower and geranium were detected in anthocyanins of Ziyan. And delphinidin content was the highest among them. Kenyan researchers [16] analyzed the components of anthocyanin in native purple varieties, and detected cyanin - 3-O-galactosin, cyanin - 3-O-glucoside, delphinium, cornflower, pelargonidin, paeoniflorin and malvidin, among which Malvidin is the most abundant.

5.2. Analysis of the Physiological Activity of Anthocyanin

Nowadays, anthocyanin is widely used in skin care [28], food [29] and medicine [30]. Previous study has found that anthocyanin has a variety of physiological activities such as antioxidant, anti-cancer, protect eyesight, relieve visual fatigue [31]. Therefore, the research on anthocyanin has always been a hot topic.

Huang reported that the antioxidant activities of proanthocyanidins and total ketones are stronger than the positive control VE through FRAR and DPPH methods [32], which indicates that proanthocyanidins and flavonoids have strong antioxidant capacity. A foreign research team [33] found that the antioxidant activity of purple chrysanthemum tea was significantly higher than that of yellow chrysanthemum tea through the DPPH free radical scavenging experiment, and that the antioxidant capacity of purple chrysanthemum tea increased with the increase of anthocyanin content. Chinese researchers [34] studied the effect of anthocyanins on the expression and production of BMDM-related pro-inflammatory and anti-inflammatory cytokines in mice and found that anthocyanins can inhibit TNF-α, IL-6, IL-1β, McP-1, INOS and other M1 type macrophage markers secretion, which indicates that anthocyanins have anti-inflammatory effects. Bai. [35] reported that the anthocyanins (BSCA) in black bean hulls have the most significant effect on inhibiting liver cancer Hep G2 cells by detecting cell proliferation experiments using MTT and EDu methods. In addition, BSCA can inhibit the JAK2/STAT3 signaling pathway to cause hepatoma Hep G2 SMMC7721 cells to undergo apoptosis, indicating that anthocyanins have anti-cancer effects and provide a theoretical basis for the development of natural anti-tumor drugs with no toxic side effects.

5.3. Analysis of Metabolic Accumulation Pathways and Gene Expression of Anthocyanin

The purple formation mechanism of tea tree is mainly anthocyanin accumulation, but the specific biochemical reaction process is not clear yet. Compared with tea tree, the differential genes identified by purple tea tree are mainly involved in 24 biochemical processes [36], including phenylpropanoid metabolism, flavonoid metabolism and glycolysis pathway [37-38]. It is speculated that the enhancement of phenylpropanol metabolism and glycolysis pathway and the improvement of acetyl-COA provide the precursor for anthocyanin synthesis and promote its production. The enhancement of some material components in tea trees can be used as accelerators and inducers for anthocyanin accumulation in this process, such as ATP-binding cassette transporter 9 (ABC transporter), brassinosterol, phenylalanine lyase (PAL), cinnamate acid carboxylase (C4H) and 4 - coumaric acyl CoA ligase (4CL), sucrose synthase [37-40].

Tang et al. [41] used real-time fluorescence quantitative PCR to analyze the expression differences of phenylalanine ammonia-lyase (PAL) gene family members in different tea tree varieties (lines) with different leaf colors. The expression levels of CsPALa, CsPALc and CsPAL3 (CsPALe) genes in purple tea trees were significantly higher than those in conventional green tea trees and albino tea trees, and the darker the purple leaves are, the higher the relative expression levels will be. The results indicated that the up-regulated expression of CsPALa, CsPALc and CsPAL3 (CsPALe) genes may promote the synthesis and accumulation of anthocyanin in tea plants, which laid a foundation for the cultivation of tea plants with high anthocyanin content. Wang et al. [42] identified the relative and absolute quantification (iTRAQ) method of the protein in Ziyun tea by isobaric labeling. The analysis showed that 20 differentially expressed proteins in Rhododendron tea are related to the regulation of anthocyanin metabolism. And the content of anthocyanin synthase, UDP-glucosyl transcriptase, adenosine band (ABC), transcription factors bHLH and HY5, bifunctional 3-dehydroquinine dehydratase and chorismate mutant enzyme are higher than those of green leaves. That lignin short was found to be reduced due to the lower abundances of cinnamoyl CoA reductase - 1, peroxidase and laccase - 15 June, Which rationed in increase of intermediates flow into anthocyanin synthesis pathway. Thus, this promoted the accumulation of anthocyanin in purple tea. UGTs cloned by Wu Hualing et al. [43] with PACE technology. And it showed remarkablely higher expression levels in purple tea varieties than in green tea varieties. The UGTs could catalyze the reaction between UDP-galactose and myxotone. The results contribute to clarifying what a role UGTs play in anthocyanin synthesis pathway.

Li et al. [44] observed that the contents of asterin, anthocyanin and phenanthrene in leaves treated with ultraviolet light (UV-A, UV-B and UV-AB) were significantly higher than that of leaves treated with white light, which provide references for planting new varieties of tea trees rich in anthocyanins. The enzyme activities of Chalcone synthase (CHS), flavonoid 3',5'-hydrogenase and anthocyanin synthase (ANS) were significantly increased under UV treatment, but the activity of anthocyanin reductase was decreased, which transferred the metabolic flux to anthocyanin biosynthesis. Li et al. [38] observed that the activities of sucrose synthase, acetyl-coenzyme and anthocyanin in Zijuan tea were higher than those of green tea leaves by analyzing the leaf transcriptome of Rhododendron tea using the Illumina HiSeq 2500 platform. This is because that sucrose synthase provides intermediate hexose and glucose for the synthesis of anthocyanins, and acetyl-CoA promotes the accumulation of anthocyanins by up-regulating the cytoplasmic acetyl-CoA carboxylase (ACCase) gene of azalea tea, which provides more basis for our study on the regulation of anthocyanin synthesis and metabolism in tea tree.

6. Analysis of Other Components of Purple Tea

Studies by Chinese scholars have shown that there is no significant difference in the polyphenol content of purple tea, but the content of total catechins is lower, and the content of anthocyanins is higher compared with Fuding white tea. There was a negative correlation between the anthocyanin and total catechin in purple tea [9, 45, 46], anthocyanins and catechins may be all in flavane - 3, 4 - glycol as raw material for synthetic biology, Indian scholar also came to a similar conclusion [18]. However, Kerio et al. [15, 16] came to the opposite conclusion in the study of purple tea in Kenyain, the study of Kenya's purple tea reported that the content of anthocyanins is similar to catechins (only includes C, EC, EG, ECG and EGCG). In addition, researchers from India and Kenya have found repeatedly high gallic acid (GA) levels in purple tea [15, 16, 18].

The content of caffeine, theanine and free amino acids in purple tea is lower than that in the green tea in the study in China and India, which may be due to the abnormal accumulation of anthocyanins in the tea which inhibits the synthesis of other substances [9, 18]. Some purple varieties meet these conclusions. According to the report of the Kenyan Research Journal, most varieties have higher theanine content than standard varieties, but have decreased caffeine content. Among them, TRFK 73/4 has higher theanine and catechins. [15, 16].

7. Physiological Activity of Purple Tea

Purple tea has attracted the attention of researchers not only for its unique color and taste changes, but also for its biologically active ingredients. The content of anthocyanins, tea polyphenols, catechins and flavonoids in purple tea is much higher than that of green tea, which makes purple tea products have good antioxidant and anti-cancer effects.

7.1. Free Radical Scavenging and Antioxidant Function

In recent years, plant extracts with antioxidant effects have received more and more attention due to their safety, high efficiency, stability, controllability and non-toxicity. It is an inevitable trend to find effective, cheap and non-toxic natural antioxidants from plants.

The main chemical component of Purple tea is anthocyanin, which has the antioxidant structure common to flavonoid compounds [30, 47, 48], and has strong free radical scavenging and antioxidant functional activity. Liu et al. [49] studied the antioxidant components and activity of extracts of Fuding White tea, Subscroll tea, Baiye No. 1 tea and Chinese yellow tea by simulated digestion in vitro. The results showed that the content of proanthocyanidins was the highest and ABTS free radical scavenging ability was the strongest. Lv [2] et al. proved that purple tea has stronger antioxidant activity than other varieties by measuring the DPPH free radical scavenging capacity, ABTS free radical scavenging capacity, iron reducing antioxidant capacity (FRAP) and cellular antioxidant activity (CAA) of purple tea products, and proved that the total anthocyanin content and DPPH antioxidant activity are positively correlated. Dai [50] compared purple tea anthocyanin extracts with antioxidants BHA and BHT commonly used in foods on this basis. The experimental results showed that the anthocyanin extract of rhododendron tea had the best ability to capture DPPH free radicals, but the ability to remove ABTS free radicals and the antioxidant ability of FRAP was slightly inferior to that of BHA. Scholars at home and abroad [51-56] aiso got the conclusion that purple tea has strong antioxidant activity in a similar experiment. Liu et al. [57] showed that the free radical scavenging ability and antioxidant activity of rhododendron tea were also different after different processing. Among them, the total antioxidant ability of PPH radical scavenging by steaming was the strongest, and that of superoxide anion scavenging by drying green was the strongest. Pt and Pg in anthocyanin, My and Qu in flavonoids and tea polyphenols were positively correlated with antioxidant activity, while the content of tea polyphenols was negatively correlated with superoxide anions. It provides scientific basis for further exploration of antioxidant active substances in rhododendron tea.

7.2. Anticancer Activity

Purple tea can achieve its anticancer purpose mainly through anti-free radical, inducing apoptosis of cancer cells and immune stimulation [56-58].

Joshi et al. [59] studied the gene expression analysis of anthocyanin biosynthesis pathway encoding enzyme LAR, CHS, F3H, DFR and ANS in normal and purple buds. The results showed that the content of anthocyanin in new purple tea iHBT-269 was higher than that in common purple tea, and the anthocyanin AN (1-4) and crude extract AN (5) had cytotoxicity on C-6 cancer cells. It has good apoptotic inducing activity and immune stimulating potential when its relative fluorescence unit (RFU) is 200lb /ml, showing the antioxidant and anticancer activity of purple tea. It contributes to the development of anthocyanin rich tea clone nutrition products. Hsu et al. [17] showed that purple tea anthocyanin extract (PTE) could inhibit the proliferation of COLC320DM and HT-29 by blocking the cell cycle process at the CO/GI stage and inducing apoptosis. And PTE induces cell cycle arrest by reducing the expression of cyclin E and cyclin D1 in COLC320DM and by upregulation of cyclin dependent kinase inhibitors p21 and p27 in HT-29. Lin et al. [60] also confirmed its anti-cancer effect, and reported that anthocyanins can help accelerate the induction of cancer cell apoptosis during chemotherapy, thereby reducing radiation dose. Joshi et al. [56] observed C-6 by adding a purple tea anthocyanin extract and using fluorescence staining, suggesting that the extract could not only induce apoptosis of cancer cells, but also stimulate the immune potential to accelerate the elimination of cancer cells. Tian et al. [61] reported that anthocyanin significantly inhibited the growth of HCT-15 and McF-10A in colon cancer cells in the study on anti-cancer activity of the extracts from Ziyan tea. Ziyan tea has stronger anticancer effect than ordinary tea when studying the effect of anthocyanin on HepG2.

7.3. Anti-inflammatory and Antibacterial

Purple tea has the effect of antibacterial and anti - inflammation. Zhang et al.[62-65] documented that VY-E, an anti-inflammatory active extract of Diethyldipine, could improve the degree of acute inflammation swelling, control the production of Prostaglandin E2 (PGE2) in inflammatory tissues and inhibit the release of NO. VY-E may achieve anti-inflammatory effects by inhibiting the expression of pro-inflammatory cytokine genes. Li et al. [66] reported that compounds isolated from rhododendron tea, including kaolin (1), (-) -epicatechin - 3-O-gallate ester (3), myricetin (4), quercetin (6), epigallocatechin gallate ester (8), 3-O-gallate quinine (9), small ephedrine (10), 1, 4, 6-3-O-gallate -β -D-glucose (11), had significant anti-inflammatory activity. Gao et al. [67] also showed that the extract of rhododendron tea has anti-inflammatory and anti-proliferative effects in vitro, which provides potential natural resources for the development of functional tea beverages.

7.4. Other Functions

Shimoda [68] has shown that purple tea extract (PTE) can induce the reduction of fat accumulation in mice, inhibit the serum and liver weight, abdominal fat and triglyceride levels through animal studies. Rashid et al. [69] studied the effect of Kenyan purple tea anthocyanins on the antioxidant activity of the brain, and proved that Kenyan purple tea anthocyanins can enhance the antioxidant capacity of the brain through the blood-brain barrier. Ochanda S O et al. [70] investigated the effect of purple tea fortified alcoholic beverage on antioxidant capacity and liver function in white Swiss mice， the result demonstrated that purple tea fortified alcoholic beverage could reduced liver alkaline phosphatase ALP, supplemented antioxidants and increased liver albumin, and improved the nutritional status of mice. Lv et al. [21] analyzed the ECCG3" Me component in fresh leaves of purple tea, which is more easily absorbed by human body. Shen et al. [71] reported that purple tea extract has a significant inhibitory effect on tyrosinase, which can be used for the development and utilization of drugs for the prevention and treatment of various pigment diseases such as melanoma. It laid the foundation for the application of purple tea extract as a natural whitening active substance in whitening skin care products and functional foods.

8. Conclusion

This article summarizes the effects of the types and processing methods of purple tea on the quality of purple tea, the discoloration mechanism of purple tea leaves, and the chemical components and physiological activities of purple tea. To sum up, purple tea has been studied and applied in many countries [73-78], but there is still a large space for research and development in many aspects. The author believes that there are mainly the following aspects: (1) The difference of geographical environment has a significant impact on the internal composition of purple tea;(2) Different processing methods lead to great differences in the quality of Purple tea. Therefore, the processing technology can be improved to develop high-quality new products to meet the market needs;(3) Further study the interaction of components of purple tea tea soup to enhance the stability of anthocyanin in tea soup [78, 79]; (4) Currently, there are few varieties of purple tea, so it has great potential in variety breeding. The screening and cloning of purple gene is an important direction in the future research on purple tea of tea tree;(5) Purple tea has its own uniqueness in the process of growth. The management technology of purple tea garden is studied to fully express the advantages of purple tea and further improve the yield and quality of purple tea;(6) According to the preliminary determination by vanillin sulphuric acid method, the proanthocyanidin is significantly higher than that of ordinary tea [80, 81], but its structure and function are still unclear;(7) Conduct in-depth research on the biological activity of purple tea and develop new products, such as new tea drinks, tea health products, cosmetics and drugs.

In the 21st century, people are more and more inclined to the healthy and unique consumer products, so the international research upsurge of natural functional food is being set off. At present, Yunnan purple tea has formed a certain scale and has been introduced in a certain number throughout the country [82, 83]. It is believed that the use of purple tea will bring the tea industry to a new stage.

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