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Effects of drying methods on the phytochemicals contents and antioxidant properties of chrysanthemum flower heads harvested at two developmental stages

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ABSTRACT

The dried flower head of chrysanthemum is a Chinese medicine, and also used as functional food material. The effects of different drying processes on the bioactive chemical contents and antioxidant activities of 'Xiaobaiju' and 'Taiju' were examined. Two kinds of harvest materials were handled with sun-dried, shade-dried or oven-dried at low, middle and high levels. An HPLC (high performance liquid chromatography) method was used to simultaneously quantify eight bioactive compounds. The antioxidant activities were assayed *in vitro*. The results showed that oven-drying had faster drying rate. The drying processes had significant effects on the contents of bioactive constituents and antioxidant properties. The appropriate drying process was suggested to be oven-dried at 60 °C for 'Xiaobaiju' and 70 °C for 'Taiju'. The sufficient dried samples were capable of scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) and O₂^{•−}, inhibiting linoleic acid oxidation. The results also implied that 'Taiju' possesses better functional effectiveness than 'Xiaobaiju'.

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Chemical compounds: Luteolin-7-O-β-D-glucoside (PubChem CID: 5280637); Apigenin-7-O-β-D-glucopyranoside (PubChem CID: 5280704); Luteolin (PubChem CID: 5280445); Acacetin-7-O-β-D-glucopyranoside (PubChem CID: 5321954); Apigenin (PubChem CID: 5280443); Acacetin (PubChem CID: 5280442); Chlorogenic acid (PubChem CID: 1794427); 3,5-di-caffeoylquinic acid (PubChem CID: 6474310); 1,1-diphenyl-2-picrylhydrazyl (PubChem CID: 74358); 2-thiobarbituric acid (PubChem CID: 2723628).

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1. Introduction

The dried flower head of *Chrysanthemum morifolium* Ramat. is called 'Ju Hua' in Chinese, and it was recorded in the first classic work of Chinese herbal medicine, "Shen Nong's Herbal Classic," about two thousand years ago. Now, it has been listed in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2010) as *Chrysanthemi Flos*. 'Xiaobaiju' (XBj) and 'Taiju' (Tj) are two kinds of processed flower heads harvested at different developmental stages from a famous cultivated breed of 'Ju Hua' (Tongxiang, Zhejiang Province, China). 'Xiaobaiju' means the ray florets are opened while the tubular florets are opened 30–70%, and 'Taiju' means the buds with the ray florets are opened while the tubular florets are not opened yet. In recent years, both XBj and Tj act as common materials for functional and healthy tea or beverage due to their unique flavour, colour and health benefits. As an herbal medicine, *Chrysanthemi Flos* has antibacterial, antioxidant, anti-inflammatory and anti-tumour properties (Akihisa et al., 1996; Duh, 1999; Ukiya et al., 2002). The functional efficacies of the herb attribute to the constituents, caffeoylquinic acids and flavonoids, such as luteolin, apigenin and their glucosides (Liu, Ong, & Li, 2013). However, the variations observed in the constituents of *Chrysanthemum Flos* can affect the quality and clinical efficacy of its products and derived preparations. Therefore, determination of the factors that contribute to the quality of *Chrysanthemum Flos* has garnered significant interest. Specifically, production of effective and consistent herbal products requires a thorough understanding of those factors that influence the quality of the herb.

Generally, drying is considered to be the crucial step in the post-harvest processes due to its importance of limiting enzymatic degradation and microbial growth while preserving the plant's beneficial properties. The drying process affects the quality of the medical herbal preparation by altering its chemical composition, active principal content and bioactivity (Li et al., 2012; Tankoa, Carrier, Duana, & Clausena, 2005; Zhu et al., 2014). Ambient air-drying is the most common technique employed, because it does not require the use of expensive equipment and the low temperatures protect against degradation of the active components. However, this drying process is slow and allows the innate metabolic processes of the plant to continue after harvest, which may lead to an adverse effect on the medicinal plants, such as colour changes or loss of active ingredients (Yabar, Pedreschi, Chirinos, & Campos, 2011). Thermal drying is another commonly employed technique for drying herbs at an industrial scale. The advantage for thermal drying is that it shortens the drying time and guarantees the consistency of the herb; however, the use of high temperatures can reduce the herbal efficacy.

Traditionally, the flower heads of *C. morifolium* are steamed after being harvested and then processed in the sun or in the shade. The process of steaming the flower heads may result in changes to the cellular structure, as well as enhanced cellular permeability and improved diffusion of moisture from the interior to the surface (Huang, Yun, & Wei, 2012). However, the drying time would last at least half a month to reach the standard level of moisture ($\leq 15.0\%$) required by the Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2010).

Natural drying processes, such as sun drying or shade drying, represent conditions that are uncontrollable, making it difficult to guarantee the safety, efficacy and consistency of an herb. In addition, no systematic comparison on the thermal and natural dried 'Ju Hua' has been reported up to now. Therefore, this study fulfils the need by characterising and optimising an appropriate drying process for this herb.

Therefore, the first objective of this study was to investigate the effects of various drying conditions on the bioactive constituents (two caffeoylquinic acids and six flavonoids) and establish the optimised drying methods for 'XBj' and 'Tj', respectively. The second objective was to compare the chemical contents and antioxidant activities of 'XBj' and 'Tj'. Taken together, these data will provide important information for understanding the quality forming of 'Ju Hua'.

2. Materials and methods

2.1. Plant materials

'Xiaobaiju' (XBj) and 'Taiju' (Tj) were collected from Tongxiang City, Zhejiang Province of China, during the harvest season in October and November of 2013. The fresh materials were taken to the laboratory within one day and immediately stored at -20°C in a refrigerator before the drying process. The samples were authenticated by Prof. Min-jian Qin and voucher specimens (No. YJ-20131001-02) were deposited in the Herbarium of Medicinal Plants in China Pharmaceutical University.

2.2. Chemicals and reagents

Reference compounds including luteolin-7-O- β -D-glucoside (LuG), apigenin-7-O- β -D-glucopyranoside (ApG), luteolin (Lut), acacetin-7-O- β -D-glucopyranoside (AcG), apigenin (Api) and acacetin (Aca) were isolated and purified in our laboratory (Zhang, Qin, & Wang, 2009). Their structures were identified with UV, IR, MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ method. The purity of each compound was determined to be over 98% by an HPLC-UV method. Standard compounds of chlorogenic acid (CA) and 3,5-di-caffeoylquinic acid (3,5-DCQA) were purchased from Shanghai R&D Center for Standardization of TCMs, Shanghai, China. Chromatography grade methanol was purchased from Merck (Darmstadt, Germany). Ultrapure water for chromatography was obtained from an ULUP-II-20T purification system (ULUP, Nanjing, China). All other reagents were of analytical grade and purchased from Nanjing Chemical Regents Co. Ltd. (Nanjing, China).

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, Tween-40, β -carotene, nitrotetrazolium blue chloride (NBT), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Co. (Shanghai, China) and used without further purification. Folin-Ciocalteu's (FC) reagent, methionine (Met) and riboflavin were obtained from Solarbio Sciences & Technology Co., Ltd. (Beijing, China). All other inorganic reagents used in these studies were of analytical grade and purchased from Nanjing Chemical Regents Co. Ltd. (Nanjing, China).

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