Effect of hot-air drying on the Content and α-glucosidase Inhibitory efficiency of Mulberry leaves Flavonoids

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Abstract: Mulberry leaves, is frequently used as a specific medication or functional diet to treat chronic disorders. The heat-sensitive ingredients, such as flavonoids, would be damaged during the drying operation. Therefore, it is improtant to investigate the drying behaviou of the mulberry leaves, but only a few studies devoted to this topic. The drying behavior of mulberry leaves during hot air drying, as well as the quantity of flavonoids in mulberry leaves under various drying parameters and their effect on α-glucosidase inhibitory efficiency, were investigated in this work. The Lewis model was used to simulate the change in moisture content of mulberry leaves during the drying process. The total flavonoids content of mulberry leaves continued to decrease along with the drying temperature and time. The flavonoids concentration in mulberry leaves was reduced by 1.372 % and 1.740 %, respectively, when dried at 40°C and 60°C for 240 minutes. Meanwhile, the lower temperature (40°C) would have a greater impact on the overall number of flavonoids in the mulberry leaves. The drying factors had a minor impact on the α-glucosidase inhibitory rate. This paper will serve as a theoretical foundation for the high-value utilization of mulberry leaf resources.

1. Introduction

Morus alba L.'s leaves, known as mulberry leaves (Mori Folium), belong to the Moraceae family. In clinical practice, it is one of the most regularly utilized Chinese medications [1]. With the development of modern monitoring and analysis technology and the continuous progress of in vivo and in vitro experimental methods, modern studies have shown that mulberry leaves contain flavonoids, alkaloids, polysaccharide, phenol acids, coumarins, amino acids, vitamins, lipids, minerals and other chemical components, which not only endowed mulberry leaves with antioxidant, lowering blood lipid, reducing myocardial oxygen consumption, lowering blood sugar, anti-tumor, and improving immunity, but also other pharmacological effects, and it has the characteristics of mild, long-lasting, and small side effects [2].Therefore, the mulberry leaf and its functional components are developed as special medicines or functional foods to treat chronic diseases and getting people out of sub-health state [3].

However, due to the high cost of storage and transportation, a large number of Chinese medicinal materials are used in the form of dried products. Under aerobic and high temperature conditions, drying will cause loss of active ingredients, especially heat-sensitive substances in raw plant materials. For example, researchers found that the content of total flavonoids in dried sea-buckthorn is much lower than freeze-dried one, and the higher the drying temperature, the greater the loss of flavonoids [4]. Therefore, how to effectively prevent the loss of active ingredients during the drying process and maintain the good efficacy of Chinese herbal medicines is an important research content to study. At present, mulberry leaves are used as traditional Chinese medicine in dry mode. However, there are few studies on the drying regularity of mulberry leaves and its active ingredients.

Nowadays, diabetes has become the one of the chronic diseases which seriously threat people’s life and health. Alpha-glucosidase inhibitors help regulate the digestion of carbohydrates, such as starch and sucrose. Because they can competitively inhibit the activity of α-glucosidase in the upper mucosa

of the small intestine, prevent the decomposition of carbohydrates into monosaccharides, and achieve the purpose of controlling the level of glucose in the blood [5]. The traditional α-glucosidase inhibitor drugs mainly include acarbose, voglibose, miglitol [6], which have some side-effects on the stomach and intestines [7]. Therefore, exploring effective, multi-targeted, safe and low-cost α-glucosidase inhibitors from natural Chinese herbal medicines has become a research hotspot [8]. The flavonoids in mulberry leaves have been proven to have a strong α-glucosidase inhibitory activity, since they can effectively control the activity of disaccharidase and can act as insulin [9, 10].

In this paper, the drying behavior of mulberry leaves during hot air drying was studied, as well as the content of flavonoids in mulberry leaves under different drying parameter and its effect on α-glucosidase inhibitory activity.

2. Materials and Methods

2.1 Materials and reagents

Mulberry leaves were purchased from Shangluo City (Shaanxi, China). Rutin standard products, α-glucosidase (from yeast) and pNPG (4-nitrophenyl-α-glucopyranoside) were purchased from Yuanye Biotechnology Co., Ltd (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), and were analytically pure without special labels.

2.2 The drying of mulberry leaves

The temperature and time are the key factors that affect hot air drying products. The improper drying temperature and time can easily cause the change of the color, texture of the products so that damage nutrients.

Water washed fresh mulberry leaves were dried under different parameters by hot air. Four drying temperatures (W), 40°C, 50°C, 60°C, and 70°C were set. The drying time under each temperature was set as 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, and 240 min.

The moisture content was measured by direct drying in an oven (101-1AB, Test Instrument Co., Ltd, Tianjin, China) and represented by the moisture content of the wet basis g/g (wet basis, wb). The related parameters of mulberry leaf hot-air drying were measured as following formula [11]:

(1) The dry basis moisture content M_t was calculated according to formula (1):

$$M_t = \frac{m_t - m_0}{m_o} \tag{1}$$

Where, M_t is the moisture content of the sample at time t, g/g (dry basis, db); m_t is the mass of the sample at time t, g; m_0 is the absolute dry mass of the sample.

(2) Moisture ratio(M_R) was calculated according to formula (2):

$$M_R = \frac{m_t - m_b}{m_0 - m_b} \tag{2}$$

Where, M_R is the moisture ratio of the sample at time t; $M_t$is the moisture content of the dry basis at the time of sample t, g/g (db) g; m_0 is the initial dry basis moisture content, g/g (db); m_b is the equilibrium dry basis moisture content, g/g (db) g.

(3) Drying rate (DR) was calculated according to formula (3):

$$DR = \frac{M_{t1} - M_{t2}}{t_2 - t_1} \tag{3}$$

Where, DR is the drying rate, g/m^2min; M_{t1} and M_{t2} are the dry basis moisture content g/g (db) at t1 and t2, respectively; $t_1$and t_2 are the drying time min, S is the area of the initial mulberry leaf raw material for drying, m^2.

2.3 Extraction and analysis of total flavonoids

The extraction and determination of total flavonoids was carried out as described by Cao et al [12] with slight modification. Weighed 0.2 g (accurate to 0.001g) mulberry leaves and mixed with 8 ml

70% ethanol solution evenly. Then, the ultrasonic extraction was conducted in a 50℃ water bath for 20 min. Collected the filtration, and filtered residues was conducted the ultrasonic extraction once again. The filtrates were combined to obtain the total flavonoids extract of mulberry leaves.

Two milliliter of the flavonoids extracts were mixed with 0.3 ml of 5% $NaNO_2$ solution and reacted at room temperature for 6 min. Then 0.3 ml of 10% Al(NO3)3 solution was added and reacted at room temperature for 6 min again. 4 ml of 4% NaOH solution was added and diluted with 70% ethanol. Then, the solution reacted at room temperature for 15 min. With rutin as the standard, the absorbance was measured at 510 nm by a UV-Visible spectrophotometer (754PC, Jinghua Technology Co., Ltd, Shanghai, China). The total flavonoid content was calculated according to the standard curve.

2.4 Assay the of α-glucosidase inhibitory rate

This experiment was carried on a 96-well plate [13, 14]. Four groups were set in the experiment, blank group, control group, sample blank group, and sample group. The reagent was added to 96-well plate according to the volume and order as described in Table 1. Firstly the solution was placed at 37℃ for 15 min after adding α-glucosidase. Then, the substrate pNPG was added and the solution was placed at 37°C for 30 min. Finally, 100μL of 1mol/L Na_2CO_3 was added to terminate the reaction. Then, the absorbance was measured at 405 nm using a microplate reader (MB-96B, Chenghuai Technology Co., Ltd, Suzhou, China). The concentration of the inhibitor required for inhibiting 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50}.

Table 1. Measurement of α-glucosidase inhibitory rate of each group.

Reagent	Blank group (B)	Control group (C)	Sample Blank group (SB)	Sample group (S)
PBS buffer (μl)	80	70	60	50
Flavonoid extracts (μl)	0	0	20	20
α-glucosidase (μl)	0	10	0	10
	Mixed evenly and reacted at 37℃ for 15 min			
pNPG (μl)	20	20	20	20
	Mixed evenly and reacted at 37℃ for 30 min			
Na_2CO_3 (μl)	100	100	100	100

The inhibition rate of the inhibitor to α-glucosidase was calculated as follows:

$$\alpha - \text{glucosidase inhibition rate } (\%) = \frac{(A_C - A_B) - (A_S - A_{SB})}{A_C - A_B} \times 100\% \quad (4)$$

Where, A_B is the absorption of the blank group, A_C is the color of the control group, A_S is the color of the sample group, A_{SB} is the color of the sample blank group.

3. Result and analysis

3.1 The effects of drying parameters on the drying characteristics of mulberry leaves

Drying is a key step in the long-term storage and is very important for usage of plant materials. Under different drying methods, there are significant differences in the water losing process with different raw materials. Hot air drying, as a simple and easy-to-operate drying method, has been widely used in industry. However, high-temperature aerobic drying method, such as oven drying, spray drying, infrared drying, is easily to cause the loss and oxidation of heat sensitive substances. Therefore, it is very important to study the impact of drying parameters on the active ingredients of raw materials.

This paper focused on the drying characteristics of fresh mulberry leaves under hot drying method. The moisture ratio of mulberry leaves was measured by using hot drying method with temperature ranging from 40℃ to 60℃. The final moisture ratio at 240 min decreased with the increase of temperature. Within the first 15 minutes, moisture ratio at each temperature dropped dramatically,

especially at the highest temperature 60℃. However, as the mulberry leaves loss moisture the rate of drying slows down.

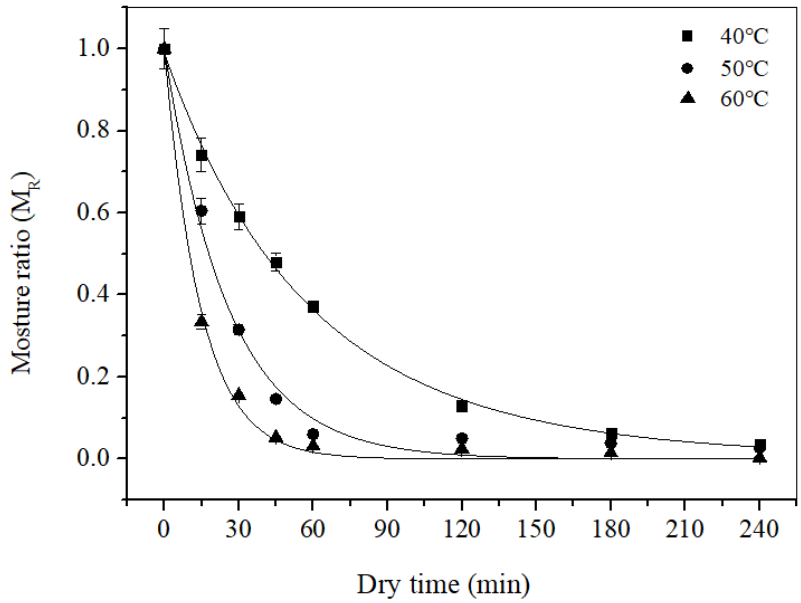

Fig 1. Drying curve of mulberry leaves dried under different parameters.

3.2 Simulation of mulberry leaf drying process

Drying is a moisture removal technique that used for high moisture content plants preservation. The drying process of plants is an extremely complex process of mass and heat transfer, which is closely related to the characteristics of raw materials and drying parameters. The purpose of reducing moisture content for materials is to inhibit the growth of microorganism, enzymatic reactions and other reactions [15]. It also can be sued for preserve the nutrients in the materials. Mathematical modeling can be used for illustrating the drying process under different drying conditions. Nowadays, there are many drying models have been applied to investigate the variation of moisture content of raw materials during the drying process, such as, Lewis model, Page model, SLogistic1 model, cubic polynomial model, Diffusion approximation model, Two-term model [16-19]. However, there are few simulation that focus on the drying process of mulberry leaves.

Table 2 shows the fitting models of the M_R and D_R of MLs at different temperatures and their corresponding parameter values. The values of coefficient of determination R^2value and the root mean square error (RMSE) are used as indicators to evaluate the effect of the fitting model. Generally, when the R^2 value was in the range of 0.900~1.000, the smaller the RMSE value was, the better was the effect of the fitting model obtained [16]. The results indicated that the exponential model fit the moisture ratio of MLs well, and the fitting R^2 values were all above 0.99. It can be seen that Lewis model can fit the moisture ratio changes during the drying process of mulberry leaves well with a relative greater R^2 of 0.99 and relative lower RMSE. Therefore, Lewis model is used for mulberry leaves drying process indication.

Table 2. Fitting formula of drying curve by different drying methods.

		40℃	50℃	60℃
M_R	Fitting model	Lewis model	Lewis model	Lewis model
	Fitting model	$M_R=e^{(0.0165\ x)}$	$M_R=e^{(0.039\ x)}$	$M_R=e^{(0.068\ x)}$
	R^2	0.998	0.991	0.997
	RMSE	7.875×10^{-5}	5.069×10^{-4}	1.463×10^{-4}

RMSE= (Residual Sum of Squares/Number of Points) ^0.5

3.3 The changes of total flavonoids in mulberry leaves during drying process

Flavonoids are a kind of important active ingredient in mulberry leaves, which have biological and pharmacological effect such as antioxidative, anti-inflammatory, and antihyperlipidemic [20, 21]. Owning to heat-sensitivity, these flavonoids are easily to be degraded at high temperatures and consequently would be significantly reduced the antioxidant activity of mulberry leaf [22]. The results of this paper indicated that with the prolonging of drying time, the total flavonoids content in mulberry leaves at each temperature all decreased (Fig.2). When dried at 40℃ for 240 min, the flavonoids content in mulberry leaves was decreased by 1.372 mg/g, which was 1.740 mg/g when dried at 60℃ for 240 min. It was interesting that, the flavonoids content when dried at 40℃ was decreased faster than that dried at 60℃, since when dried at 40℃ and 60℃ for 60 min, the flavonoids content of mulberry leaves was decreased by 0.911 mg/g and 1.417 mg/g, respectively. It was indicated that the lower temperature would affect the total amount of flavonoids in the mulberry leaves more significantly.

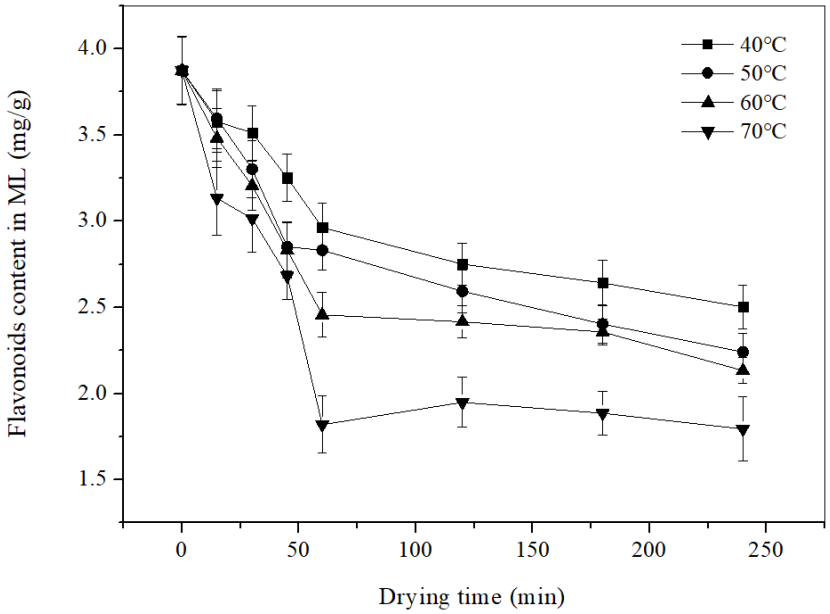

Fig 2. Effect of drying parameters on the total flavonoids content in mulberry leaves.

3.4 Effects of drying parameters on α-glucosidase inhibitory of mulberry leaves flavonoids

Alpha-glucosidase inhibitor is known to be helpful for diabetic since it can decrease the glycosylated hemoglobin levels and insulin concentration so that reduce glucose variability [20, 23]. In this paper, the difference of α-glucosidase inhibitory activity of flavonoids extracted from mulberry leaves that dried under different drying parameters were studied by in vitro evaluation (Fig.3). The results indicated that the overall inhibitory rate decreases with the decreasing of flavone concentration. At the same temperature (40℃), the longer drying time (from 30 min to 60 min), and the lower α-glucosidase inhibitory rate was obtained. However, under the same drying time (60 min), the higher drying temperature was (40℃ to 50℃), the lower α-glucosidase inhibitory rate was obtained. The ExpDec1 model was excellently fitted the relationship between concentration of flavonoids extracted from dried mulberry leaves and α-glucosidase inhibitory rate (Fig.3), since the R^2 of each fitting equation was both higher than 0.99 (Table 3). The IC_{50} of α-glucosidase by flavonoids extracted from the dried mulberry leaves was just variated slightly, which mean that the drying parameters tested in this paper hardly affect the α-glucosidase inhibitory efficiency of mulberry leaves flavonoids.

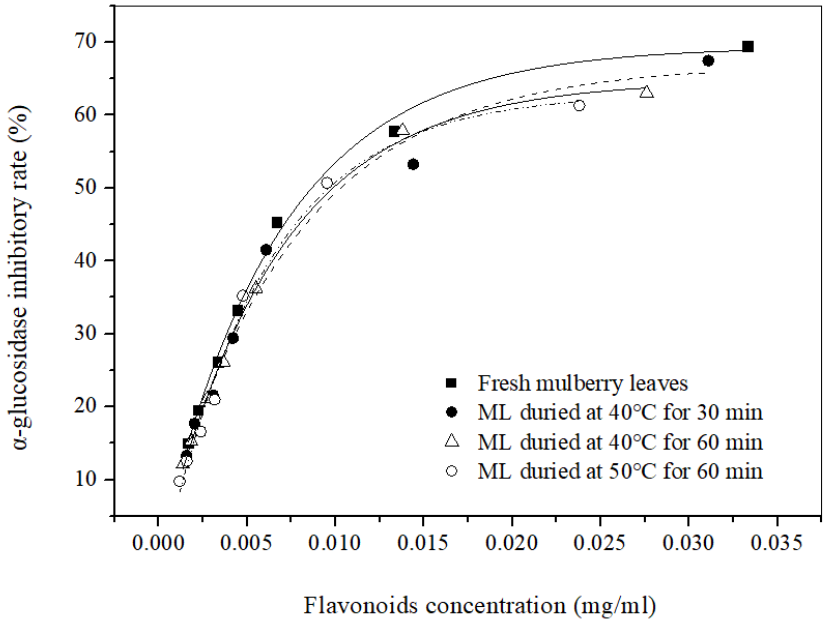

Fig 3. The α-glucosidase inhibitory rate of flavonoids extracted from mulberry leaves dried at different parameters.

Table 3. The α-glucosidase inhibitory efficiency of flavonoids extracted from different mulberry leaves.

Flavonoids*	Fitting equation	R^2	IC_{50} (ug/ml)
F_0	Y=-69.55*exp(-x/0.0072)+69.715	0.993	9.077
$F_{40/30}$	Y=-68.68*exp(-x/0.0071)+68.078	0.991	9.477
$F_{40/60}$	Y=-66.45*exp(-x/0.0069)+65.764	0.998	9.927
$F_{70/60}$	Y=-67.03*exp(-x/0.0059)+63.01	0.991	9.673

*F_0, $F_{40/30}$, $F_{40/60}$, $F_{70/60}$, represent as the flavonoids extracted from the fresh mulberry leaves, the mulberry leaves dried at 40℃ for 30 min, the mulberry leaves dried at 40℃ for 60 min, and the mulberry leaves dried at 70℃ for 60 min, respectively.

4. Conclusion

Owning to the enrichment of active ingredients that have significant α-glucosidase inhibitory efficiency, such as alkaloids, flavonoids and polysaccharose, mulberry leaves have broad prospects for resource development and utilization. The effects of drying parameters on the content and α-glucosidase inhibitory efficiency of flavonoids in mulberry leaves were investigated. The results indicated that under different drying temperatures, the moisture ratio of mulberry leaves decreased exponentially with the drying time. During the drying process, the total flavonoids content of mulberry leaves kept decreasing with the increase of drying temperature and the extension of drying time. However, the α-glucosidase inhibitory rate of the flavonoids of mulberry leaves was slightly affected by the drying conditions.

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