International Core Journal of Engineering Volume 7 Issue 11, 2021
ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

Compare the influence of different drying parameters on the main chemical components in mulberry leaves

Fangting Yao[1, *], Shijia Lin[2, a], Boyu Lu[3, b], Xingyu Ren[4, c], Zijing Meng[5, d]

[1] University College London, LON, UK

[2] Hangzhou High School, Hangzhou City, Zhejiang Province, China

[3] The Affiliated High School to Hangzhou Normal University, Hangzhou City, Zhejiang Province, China

[4] Hangzhou Foreign Languages School, Hangzhou City, Zhejiang Province, China

[5] Soochow University, Soochow City, Jiangsu Province, China

*Corresponding author: uczlfy6 @ucl.ac.uk, [a] 310342661@qq.com, [b] 2650149432@qq.com, [c] 13758175316@qq.com, [d] 1260977118@qq.com

These authors have contributed equally to this work

Abstract

Mulberry leaf is a traditional Chinese medicine material. This is because it contains flavonoids and other nutrients. After these ingredients are extracted and enriched, they can be used for the synthesis of hypoglycemic drugs. Drying technology has an important effect on the enrichment of nutrients in plants, which requires research to find suitable conditions for drying mulberry leaves. With the domestic emphasis on the development of Chinese medicine, more and more researches began to focus on the development of drying technology.

Keywords

Mulberry leaf, drying technology, polysaccharide, flavonoid, alkaloids.

1. Introduction

Mulberry leaves are the leaves of the mulberry plant of the Mulberry family. The traditional processing methods are: washing and removing the handle, cutting, drying, frying or steaming, and making mulberry leaves into tea before packaging. For a long time, most of the studies and applications on the active components and pharmacological effects of mulberry leaves in China were carried out with dry products. Modern research believes that fresh herbs are not processed, maintain the natural state and molecular structure, so the effective component content is high, good efficacy. In addition, it has more significant effects than liver and spleen in improving human immune function, lowering blood glucose (Liu et al., 2004), anti-tumor, antibacterial and anti-platelet aggregation activities, greatly reduce the risk of having diabetes[2] (Kumar et al., 2011). However, the existing processing of mulberry leaves (including food and medicine) is mainly based on dry products. Among the existing preservation technologies, freeze-drying is the best, but the equipment requirements and costs are high. The second is air drying, but the industrial application requires a large area of the plant and the drying time process; Drying due to higher temperature and uneven heating, leading to greater loss of heat-sensitive substances. In addition, in order to prolong the storage life of mulberry leaves, the above drying methods mostly dry mulberry leaves to anhydrous state, which inevitably leads to

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

the great difference between dry and fresh mulberry leaves in terms of chemical composition, drug effect and other aspects.

This research will argue that by studying the change rule of chemical composition in fresh mulberry leaves with temperature under different drying temperatures, the best parameters of fresh mulberry medicinal materials preservation and storage were screened, and the quality preservation and storage period of fresh mulberry leaves was prolonged under the premise of the curative effect of fresh mulberry leaves. Therefore, the limitations of the traditional dry mulberry leaf and the traditional fresh mulberry leaf in the pursuit of fresh and waterless state were broken as well as the best balance of the "fresh" state in the drying process of fresh mulberry leaf was explored.

2. Materials and methods

2.1 Materials

Ultraviolet–visible spectrophotometry (JASCO, V-730 UV-Visible Spectrophotometer). Rotary evaporator (Lab Tech). Pulverizer (retsch). Water bath (Digital Water Bath with In-Built Shaking Tray, 22 Litres thermoline. scientific). Freeze Dryer (Lyovapor^{TM} L − 300, *buchi*).

2.2 Effects of hot air drying (HAD) and heat treatment on the preservation and storage of mulberry leaves

The temperature and time of hot air drying are the key factors affecting the quality of products. Improper drying temperature and time will easily cause damage to the color, texture and nutrients of the product. Thus, in this experiment, mulberry leaves were dried at different temperature and time.

In this case, before drying, the sample is made into slurry with a mixer, and then dried with different parameters. The drying temperature (W) is set to two levels, respectively: W1: 40 ℃; W2: 50 ℃. Four levels of drying time (T) are set as follows: T1: 15 min; T2: 30 min; T3: 45 min; T4: 1 h.

2.3 Moisture Assay

In a measuring bottle of known weight, weigh 5-10g (accurate to 0.001 g) of a freshly cut sample, place it in an oven, open the lid, and bake at 60-70 ℃ for 6 hours. Then the temperature is raised to 100-105 ℃ for 3-4 hours, keeping the lid closed. Next, take out the sample and place it in a desiccator, cool to room temperature (about 30 minutes), and weigh. Finally, bake for another 2h, cool and weigh until constant weight (the difference between the two weighings is not more than 3 mg)

Calculation formula: moisture%= (Fresh sample weight- Dry sample weight)/ Fresh sample weight*100 (1)

2.4 Extraction and analysis of total flavonoids

The mulberry leaves treated with different temperatures and heating times were added with 70% ethanol solution, and after standing at room temperature for 2 hours, the filtrate and the residue were separated by suction filtration. The filtrate is used for the determination of total flavonoids, and the filter residue is dried for later use. (Bergeron et al., 2005)

In this experiment, the determination of flavonoids uses rutin as the reference substance. The specific method is as follows: firstly, precisely measure 0, 1, 2, 3, 4, and 5ml of 0.2 mg/mL rutin solution and place them in 10ml volumetric flasks, add 0.3ml of 5% sodium nitrite solution, shake well, and let stand for 6min. Then, add 0.3ml of 10% aluminum nitrate solution, shake well and let stand for 6min, thirdly, add 0.3ml of 10% aluminum nitrate solution, shake well and let stand for 6min. After that, add 4ml of 4% sodium hydroxide solution, dilute with 70% ethanol to the 10ml mark, shake well, standing for 15min, the absorbance was measured at 510 nm. Draw a standard curve with absorbance (A) as the ordinate and reference substance concentration (C) as the abscissa.

Precisely measure 2 ml of mulberry leaf filtrate and place it in a 10 ml measuring flask. Add 0.3 ml of 5% sodium nitrite solution and shake well and let stand for 6 min; add 0.3 ml of 10% aluminum nitrate solution, shake well, and let stand for 6 min. Add 4ml of 4% sodium hydroxide solution, dilute with 60% ethanol to a constant volume scale, shake well, and let stand for 15min. With 60% ethanol

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

solution as a blank, measure the absorbance at 510nm to calculate the content of total flavonoids. Three parallel experiments were conducted for each group of experiments.

$$Y=[(C\times V_2)/V_3\times V_1]/M\times 10^{-3}\times 100 \quad (2)$$

Where: Y is the yield of total flavonoids in the sample %; C is the mass concentration of flavonoids in the standard curve in mg/mL; V1 is the total volume of the test solution in mL; V2 is the constant volume of the test solution in mL; V3 is the test solution volume in mL; M is the weight g of the mulberry leaf sample.

2.5 Extraction and analysis of total polysaccharides

2.5.1 Hot water extraction

The polysaccharides were extracted using hot water as a solvent (Liu & Huang, 2019). Take 0.5 g of mulberry leaves dried in the above-mentioned different ways into a 50 mL centrifuge tube, add 20 mL of distilled water, and extract at 80 ℃ for 2 h. After cooling, the extract and residue are separated by suction filtration. And dilute the extract to 50ml for use. Dry the filter residue for later use.

2.5.2 Polysaccharide assay method

2.5.2.1 DNS reagent preparation

Take 3.15g of 3,5 dinitrosalicylic acid, dissolve it in 131 ml of 2M NaOH solution, add it to 250 ml of hot water solution containing 91.0 g of potassium sodium tartrate, stir to dissolve, then add 2.5 g of phenol. Mix with 2.5 g of sodium sulfite, fully stir, dissolve, cool and dilute to a 500 ml brown volumetric flask for storage, and store at room temperature for 1 week after stable use (Gusakov et al., 2011).

2.5.2.2 Hydrolysis of total polysaccharides from mulberry leaves

Precisely pipet 20.0 ml of the above polysaccharide sample solution, put it in a triangular conical flask, add 10 ml of 6M HCl solution (prepared now), seal, heat on a boiling water bath for 30 minutes, take out and cool to room temperature, adjust the pH with 6 M NaOH solution. When the value reached 8.0, centrifuged at 4000 rpm for 5 min, the residue was washed with water, the supernatant and the water washing solution were placed in a 50 ml volumetric flask, and the volume was adjusted to the mark with distilled water, and the mulberry leaf polysaccharide hydrolysate sample was obtained by shaking.

2.5.2.3Drawing of a standard curve

Precisely weigh out an appropriate amount of dry constant weight D-anhydrous glucose reference substance, add water to dissolve it to make 400 mg/L, accurately pipette 0, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 ml of glucose reference substance solution, and place a test tube with stopper. Add water to 2.0 ml respectively, add 1.5 ml DNS reagent, shake well, heat in a boiling water bath for 5 min, take it out and cool quickly, use the first sample as a blank, measure its absorbance at 520 nm, and the concentration of each sample. Make 3 copies in parallel, take the mass of glucose (mg) contained in each 3.5 ml solution as the abscissa and the average absorbance as the ordinate, draw a standard curve, and get the regression equation.

2.5.2.4 Assay of total polysaccharide content in mulberry leaf extract

Precisely pipette 1.0 ml of different hydrolysate samples (adjust the sampling volume according to the experimental results), place in a tube with a stopper, add water to 2.0 ml, add 1.5 ml DNS reagent, shake well, heat it in a boiling water bath for 5 min, take it out and cool it quickly, with 2.0 ml distilled water as the blank group. The absorbance was measured at 520 nm with an ultraviolet-visible spectrophotometer. Calculate according to formula 3.

$$\text{Polysaccharide content (\%)} = \frac{m}{\frac{m_{sample}}{250}*\frac{V_{\text{polysaccharide}}}{100}*\frac{V_{Hydrolyzate}}{50}*1000}*0.9 \quad (3)$$

where: m is the glucose content (g) calculated according to the regression equation, m sample is the sample sampling volume (g), V polysaccharide is the polysaccharide sampling volume during hydrolysis (ml), and V hydrolyzate is the volume of hydrolysate taken during color development

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

(ml)); When polysaccharides are hydrolyzed into monosaccharides, one molecule of water needs to be added for each break of a glycosidic bond, so it is necessary to multiply by 0.9 when calculating the monosaccharide content.

2.6 Extraction and analysis of total alkaloids

2.6.1 Extraction of total alkaloids

Accurately weigh 10.000 g of mulberry leaves, extract with 200 mL of 60% ethanol solution for 2 h at 60 ℃, concentrate the extract to 10 mL, add 3 times the volume of 95% ethanol, leave it at 4 ℃ overnight, and centrifuge to remove the precipitate. After the supernatant is concentrated to remove ethanol, it is dissolved in water, passed through a 732 cation exchange resin column, and eluted with 0.5mol/L ammonia water. The eluent is concentrated under reduced pressure, and the volume is fixed in a 10 mL volumetric flask as a test solution.

2.6.2 Analysis method of total alkaloids

Assay the content of alkaloids by the Reinecke's salt method (Wang et al., 2009), the details are as follows.

2.6.2.1 Preparation of standard solution and Reinecke's salt solution

Accurately weigh 4.000 g of 4-hydroxypiperidine, dissolve in distilled water and dilute to 100 mL to obtain a 4-hydroxypiperidine standard solution with a concentration of 0.0405 M. Accurately weigh 1.0000 g of Reynolds salt, add distilled water to dissolve and dilute to 50 mL, to obtain a Reynolds salt solution with a concentration of 20 g/L.

2.6.2.2 Drawing of a standard curve

Pipette 0.1, 0.3, 0.5, 0.7, 0.9 mL of 4-hydroxypiperidine standard solution with a concentration of 0.04 mol/L, add 0.05 mol/L hydrochloric acid solution to make up to 2 mL, and then add 10 g/L Reye's salt solution 1 mL , Vortex for 10 s, ice-water bath for 1.5 h, centrifuge for 5 min, rotate at 3500 r/min, dissolve the precipitate with 70% acetone solution and dilute to 10 mL, measure 4-hydroxypiperidine ammonium salt at 523 nm. The absorbance value of the acetone solution is linearly regressed with absorbance (Y) as the ordinate and 4-hydroxypiperidine concentration (X) as the abscissa to draw a standard curve.

3. Result and discussion

3.1 Changes of total polysaccharide content in mulberry leaves under different drying conditions

Table 1. The polysaccharide content in mulberry leaves and the percentage of increase in polysaccharide content dried at 40 ℃ and 50 ℃ for 15min, 30min, 45min, and 60min.

condition	polysaccharide content	Increase in polysaccharide content
40℃/15min	62.791	14.990%
40℃/30min	63.953	17.117%
40℃/45min	79.367	45.345%
40℃/60min	76.410	39.930%
50℃/15min	57.954	6.132%
50℃/30min	84.960	55.587%
50℃/45min	84.442	54.640%
50℃/60min	70.620	29.326%
fresh sample	54.606	

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

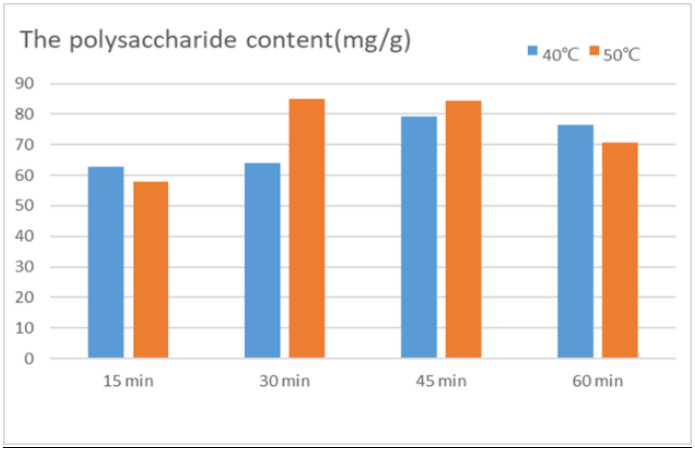

Figure 1. Graph of polysaccharide content after drying at different temperatures and times

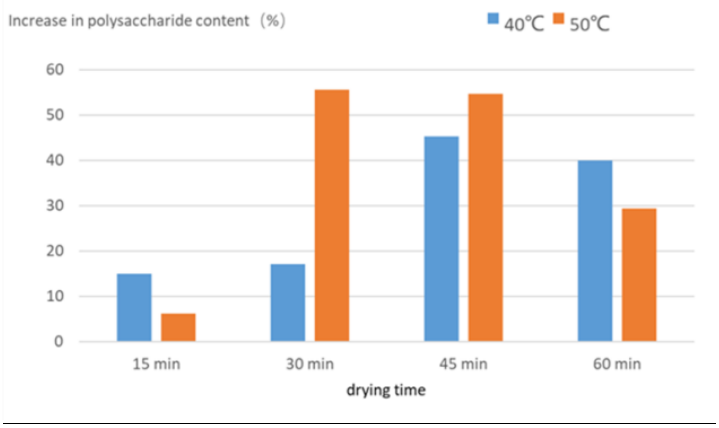

Figure 2. Graph of the growth rate of polysaccharide content after drying at different temperatures and times

It can be clearly seen from the figure that when the drying condition is 50 ℃ for 30 minutes, the total polysaccharide content obtained is the highest, with an average concentration of 84.96 mg/g. It is not difficult to find that after 30 minutes of drying, the polysaccharide content in the sample will show a downward trend as the drying time increases. In addition, in the group where the drying temperature was set to 40 ℃, the increase rate of total polysaccharide content was highest when the drying time was 45 minutes. In the 50 ℃ group, the total polysaccharide content obtained by the two groups with a drying time of 30 minutes and 45 minutes is very close.

3.2 Hanges of total flavonoids in mulberry leaves under different drying parameters

Table 2. After drying the mulberry leaves at different temperatures (40℃ and 50 ℃) for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, the flavonoid content and the loss rate of flavonoids in the mulberry leaves were obtained.

condition	Flavonoid content (mg/g)	Flavonoid loss rate (%)
40℃/15min	2.571333	33.61633
40℃/30min	2.346333	38.54033
40℃/45min	2.129	45.04367
40℃/60min	2.111	45.499
50℃/15min	2.553	34.09033
50℃/30min	2.020667	47.84
50℃/45min	1.848667	52.27933
50℃/60min	1.668	56.935
fresh sample	3.874	

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

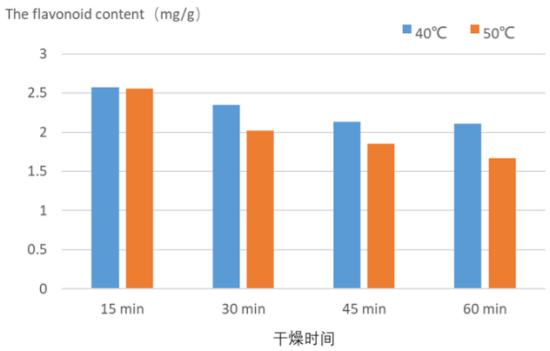

Figure 3. Graph of flavonoid content after drying at different temperatures and times

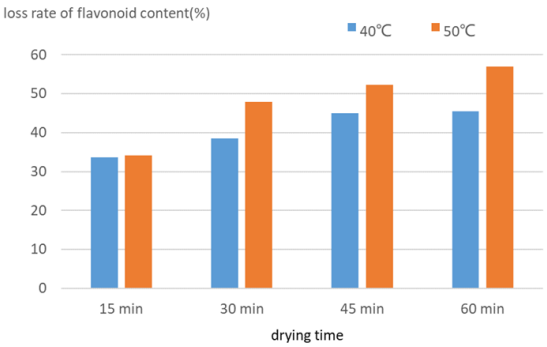

Figure 4. Graph of the loss rate of flavonoid content after drying at different temperatures and times

Firstly, it can be known from the data that the content of flavonoids in dried mulberry leaves is lower than that in fresh mulberry leaves. Secondly, comparing different experimental temperatures, it can be found that the higher the temperature, the higher the flavonoid loss rate. In the same temperature group, the longer the drying time, the higher the flavonoid loss rate.

Therefore, from these data, we can infer that the increase of temperature may cause the loss of flavonoid content in mulberry leaves. Research suggests that with the increase of temperature, the flavonoid content could show a trend of first rising and then falling (Wang et al., 2017). This trend was observed in the experimental group with a drying temperature of 40℃, although the results were not very significant. This may be caused by the insufficient number of groups designed in this experiment and the large temperature range. In further studies, the accuracy could be improved by adding more groups.

3.3 Contents of total alkaloids in mulberry leaves under different drying parameters

Table 3. After drying the mulberry leaves at different temperatures (40℃ and 50 ℃) for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, the total alkaloid content and the loss rate of that in the mulberry leaves.

condition	Total alkaloid content (mg/g)	Total alkaloid loss rate %
40℃/15min	3.9303	4.761
40℃/30min	3.569	13.525
40℃/45min	3.693	10.517
40℃/60min	3.439	16.676
50℃/15min	3.852	6.666
50℃/30min	3.614	12.430
50℃/45min	3.279	20.538
50℃/60min	3.341	19.053
fresh sample	4.127	

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

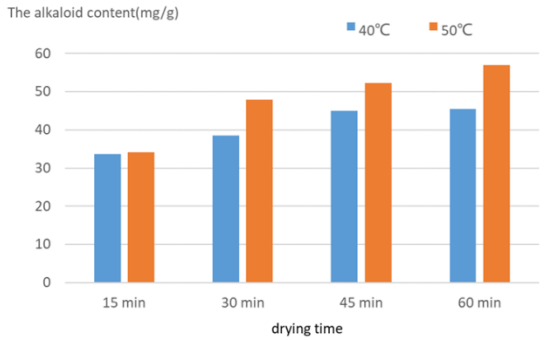

Figure 5. Graph of alkaloid content after drying at different temperatures and times

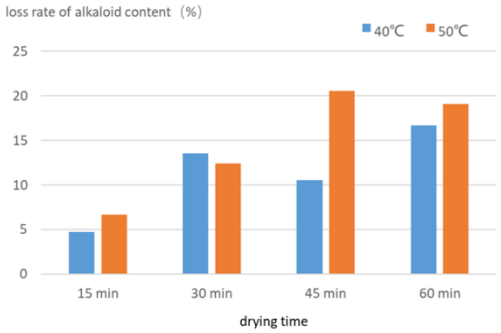

Figure 6. Graph of the loss rate of alkaloid content after drying at different temperatures and times

According to the data, the content of alkaloids in mulberry leaves is lower than that in fresh mulberry leaves after drying under different conditions, which is similar to the performance of flavonoids. In the experimental group at 40℃, the loss rate of alkaloid content did not have an obvious linear relationship with the drying time, and 60 minutes of drying time lost the most alkaloid content. In the 50 ℃ group, the alkaloid content decreased with increasing drying temperature, except that the total alkaloid content was 3.341 mg/g when the drying time was 60 minutes.

For our research, we extract flavones and alkaloids using resin adsorption. This method has been proved to be effective, simple and easy to do, and low-cost from the research “Purification of Total Alkaloids from Flower of Intermediate Peashrub by Macroporous Resin” (Zhang et al., 2017).

4. Conclusion

In this essay, the goal was to assess the optimal drying temperature and drying time of fresh mulberry leaves and to obtain the optimal drying conditions of mulberry leaves by comparing the contents of polysaccharides, flavones and alkaloids in mulberry leaves under different drying temperatures and times. According to the experimental data, under the drying conditions of 50℃, 45 minutes, the nutrient content in mulberry leaves as indicators were the highest or the loss rate were the lowest. Therefore, 50℃, 45 minutes is considered to be a suitable condition for drying fresh mulberry leaves.

Traditional drying methods for Chinese medicinal materials include sun-drying, drying in the shade and others, while modern drying methods include hot-air drying, vacuum drying, microwave drying and far-infrared drying and so on. The drying methods for processing mulberry leaves in producing areas are generally sun-drying, drying in the shade or hot-air drying. However, there are some drawbacks for the traditional drying methods. For examples, most of them heavily depend on the weather condition and also need a lot of time for the drying process. Thus, the methods should be improved in order to meet the increasing demand. For the methods we used in our research, it has many advantages that others do not have.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202111_7(11).0079

References

[1] Liu, Yinghua teng junying, Zheng Zi, Xin Zhang Yuehong, Zhang Rongxin, Liu Xinhuan, Xue Changyong Chinese Journal of Clinical Rehabilitation, September 2004 Vol.8 No. 27.

[2] Journal of Ethnopharmacology, 27(1989)243-275 Elsevier Scienntist Publishers Ireland Ltd.

[3] Kumar, S., Narwal, S., Kumar, V., Prakash, O. and Phadatare, A.G, (2011). 'alpha-glucosidase Inhibitors from Plants: A Natural Approach to Treat Diabetes', Pharmacognosy Reviews, 5 (9), pp.19-29.

[4] Wang, S. and Song, H, (2009). 'Colorimetric determination of total alkaloids in Ligusticum chuanxiong with reinecke salt', Cent. South Pharm, 7, pp.824-826.

[5] Bergeron, C., Gafner, S., Clausen, E. and Carrier, D.J, (2005). 'Comparison of the chemical composition of extracts from Scutellaria lateriflora using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction', J Agric Food Chem, 53 (8),pp.3076-3080.

[6] Liu, Y. and Huang, G, (2019). 'Extraction and derivatisation of active polysaccharides', Journal of enzyme inhibition and medicinal chemistry, 34 (1), pp.1690-1696. doi: 10.1080/14756366. 2019. 1660654.

[7] Gusakov, A.V., Kondratyeva, E.G. and Sinitsyn, A.P, (2011). 'Comparison of Two Methods for Assaying Reducing Sugars in the Determination of Carbohydrase Activities', International Journal of Analytical Chemistry, 2011, pp.1-4.

[8] Wang Dingmei; Wang wei. Qin-fen li; Mingtao Lin; Han-ting cheng; Guang-yi li; Malewen, Effect of drying temperature on the extraction rate of flavonoids from tender stem and leaves of cassava of two varieties, Journal of Agricultural Resources and Environment,2017,

[9] Zhang Yanzhen, Wang Fei. Studies on purification process of total alkaloids in Caragana [J]. Fresh Preservation and Processing,202,20(05):83-88. (in Chinese)