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# EXTRACTION OF POLYSACCHARIDES FROM THE MESOSPERM OF *GINKGO BILOBA* L. BY AQUEOUS ENZYMATIC EXTRACTION

GUOXIA WANG*, HUIYU WANG, YUZHEN YANG, CHUNGE LI,
QING LUO AND DAN SONG

*School of Life Sciences, Zhengzhou Normal University, Zhengzhou, 450044, China*

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## Abstract

Effects of enzyme dosage, solid-liquid ratio, temperature and time required for the extraction rate of polysaccharides from the mesosperm of *Ginkgo biloba* were investigated by single factor and orthogonal tests. The results showed that the highest extraction rate of polysaccharides can be up to 103.041 mg/g. when temperature was 70ºC, material liquid ratio was 1 : 15, enzyme dosage was 0.6% and extraction time was 70 min. The results demonstrated that the orthogonal experiment can significantly improve the extraction rate of polysaccharide from Ginkgo mesosperm, thereby greatly saving energy and enhancing efficiency.

## Introduction

Polysaccharides are important natural active organic substances which are widely distributed in plants, animals and microorganisms (Tan *et al.* 2017, Kang *et al.* 2010). Polysaccharides possess great medical and hygenic values mainly including the improvement of immunity, anti-aging, anti-oxidant, anti-cancer and hypoglycemic functions as well as anti-bacterial effect (Shi *et al.* 2014, Shi *et al.* 2017, Zhang *et al*. 2018, Shang *et al*. 2015, Wang *et al*. 2016). *Ginkgo biloba* belonging to Ginkgopsida, a kind of deciduous, precious relict plants can only be found in China. Leaves, flowers, fruits and woods of *Ginkgo biloba* exhibit great economic, ecological, ornamental and scientific values (Wang *et al*. 2015).

In China, *Ginkgo biloba* is featured by large cultivated area and high yield. Each year, massive testa (including both episperm and mesosperm) of *Ginkgo biloba* was discharged as agricultural wastes, causing serious pollutions and waste of resources (Xu *et al*. 2017). Currently, a great deal of research has been conducted on the development and utilization of episperm of *Ginkgo biloba* and also extensive investigation has been carried out on the extraction technologies (Wu *et al*. 2006, Mou *et al*. 2010, Wang *et al*. 2014, Cao *et al*. 2015, Xu *et al*. 2017, Wu *et al*. 2017, Lei *et al*. 2017). However, the extraction method of polysaccharides from the mesosperm of *Ginkgo biloba* has been poorly studied. Therefore, an attempt has been made to develop a method of extraction of polysaccharides from mesosperm of *Ginkgo biloba* for the first time.

## Materials and Methods

Mature Ginkgo fruits were collected from the campus of Zhengzhou Normal University in early October, 2017. After the episperm was removed, the fruits were placed in the laboratory and dried; next, the mesosperm was peeled off and placed in the air-drying oven for drying at 65ºC until the mass becomes constant. Finally, the mesosperm after drying was ground to powder in a cyclone mill, placed in the sealed bag and stored in a glass bottle. The processed mesosperm was placed in the refrigerator and stored at 4ºC. The used reagents mainly including glucose, anthranone, concentrated sulfuric acid and cellulose were all analytically pure.

*Author for correspondence: <wgxia191919@sina.com>.

This study referred to the method proposed and modified by Chen *et al.* (2017). Initially, 0.01 g glucose was weighed, dissolved into a small amount of distilled water and diluted in a bottle to 100 ml. The prepared glucose standard solutions (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 ml) were pipetted in different tubes. Thereafter, each standard liquid was diluted with distilled water to 1 ml. Using anthrone-sulfuric acid colorimetry, the absorbance of standard liquid was measured for the fitting in linear regression equation. According to measured absorbance values of the glucose standard solutions with different mass fractions, the linear regression equation was fitted as: y = 5.743x + 0.0093, with a regression coefficient of $R^2 = 0.9967$, in which y denotes the absorbance and x denotes the concentration of the glucose solution.

According to the prediction results of preliminary experiments, the solid-liquid ratio was set as 1:20. Mesosperm powder (0.5 g) was added to a beaker, and meanwhile, using a measuring cylinder, 10 ml distilled water was measured and added into the beaker. Next, the corresponding amount of cellulose was added to the mixed solution, shaken well and placed in a constant-temperature water bath for certain time; after being cooled to room temperature, the extracting solution was poured into a centrifugel tube and placed in a centrifugel machine; finally, after a centrifugation for 15 min at a speed of 4000 rpm, the supernatant was taken for further investigation.

The present experiments were first performed under the following conditions, a solid-liquid ratio of 1 : 20, a dosage ratio of cellulose of 0.5% and an extraction temperature of 50ºC. Under this condition, the effect of polysaccharide from Ginkgo mesosperm at different extraction times (50, 60, 70, 80, 90 and 100 min) were measured.

The experimental condition was then set as, a dosage ratio of cellulose of 0.5%, an extraction temperature of 50ºC and an extraction time of 60 min. Under this condition, the effect of solid-liguid ratio on the extraction of polyaccharides different solid-liquid ratios (1 : 5, 1 : 10, 1 : 15, 1 : 20, 1 : 25 and 1 : 30, respectively) were measured.

Next, the experimental condition was set as: an extraction temperature of 50ºC, an extraction time of 60 min and a solid-liquid ratio of 1 : 20. Under this condition, the effect of the dosage of cellulose on the extraction of polysaccharides from Ginkgo mesosperm under different dosage ratios of cellulose (0.4, 0.5, 0.6, 0.7, 0.8 and 0.9%, respectively) were measured .

Finally, the experimental condition was changed as: an extraction time of 60 min, a solid-liquid ratio of 1 : 20 and a dosage ratio of cellulose of 0.5%. Under this condition, the effect of the extraction temperature on the extraction of polysaccharides from Ginkgo mesosperm at different extraction temperatures (50, 60, 70, 80, 90 and 100ºC) were measured.

By analyzing single-factor experimental results, each factor was optimized and orthogonal optimization experiment was then conducted on four factors in accordance with $L_9$ ($3^4$) Table (Table 1).

**Table 1. Orthogonal experimental factors and level.**

| Level | Extraction temperature (ºC) | Solid-liquid ratio | Dosage ratio of cellulose (%) | Extraction time (min) |
|---|---|---|---|---|
| 1 | 65 | 1:5 | 0.4 | 70 |
| 2 | 70 | 1:10 | 0.5 | 80 |
| 3 | 75 | 1:15 | 0.6 | 90 |

One ml from polysaccharide extracting solution of Ginkgo mesosperm was placed in a 50 ml flask and diluted by the distilled water to 50 ml, thereafter, the absorbance of the solution was measured at OD by means of anthrone-sulfuric acid colorimetry, and the measured absorbance was substituted into the standard curve of glucose solution for calculating the extracting amount of polysaccharide.

The extracting amount of polysaccharide was calculated as:

$$\text{XX (mg/g)} = \frac{C \times V \times N}{M}$$

where XX denotes the extracting amount of polysaccharide, C denotes the concentration of the polysaccharide (mg/g), V denotes the volume of the supernatant (ml), N denotes the dilution ratio in the present experiment and M denotes the mass of Ginkgo mesosperm powder.

Statistical analysis was then performed on the present experimental data using EXCEL2007 and DPS10.5.

**Results and Discussion**

As shown in Fig. 1 different amounts of polysaccharide were extracted from the Ginkgo mesosperm after different extraction times. When the solid-liquid ratio, the dosage ratio of cellulose and the extraction temperature remained unchanged, the extraction ratio of polysaccharide first increased and then dropped with the increase of extraction time. After an extraction time of 50 ~ 80 min, the extraction ratio of polysaccharide increased significantly, which reached a maximum of 73.034 mg/g after an extraction time of 80 min. Afterwards, the extraction ratio of polysaccharide dropped with the increase of extraction time. This might be attributed to the decomposition of some polysaccharide with the increase of extraction time. Therefore, the extraction time was set as 70, 80 and 90 min for further orthogonal optimization experiment.

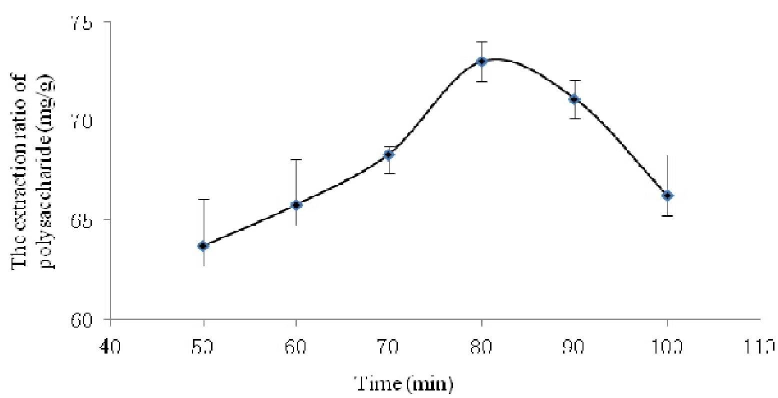


Fig. 1. Effect of the extraction time on the extraction ratio of polysaccharide from Ginkgo mesosperm.

If the extraction temperature, the dosage ratio of cellulose and the extraction time are kept unchanged, the extraction ratio of polysaccharide first increased and then dropped with the increase of solid-liquid ratio (Fig. 2). As the solid-liquid ratio increased from 1 : 5 to 1 : 10, the extraction ratio of polysaccharide increased gradually, which reached a maximum of 67.261 mg/g at a solid-liquid ratio of 1 : 10; then, as the solid-liquid ratio further increased, less polysaccharide was extracted from the Ginkgo mesosperm. Therefore, the solid-liquid ratio was set at 1 : 5, 1 : 10 and 1 : 15 for further orthogonal optimization experiment.

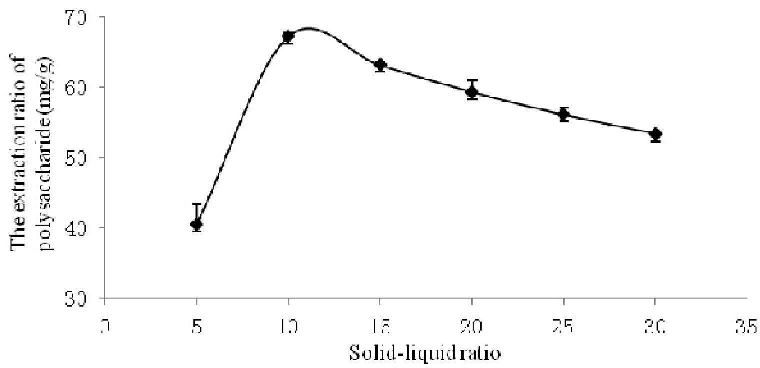


Fig. 2. Effect of the solid-liquid ratio on the extraction ratio of polysaccharide from Ginkgo mesosperm.

Fig. 3 shows that the extracting amount of polysaccharide varies with the dosage ratio of cellulose. If the solid-liquid ratio, the extraction temperature and the extraction time are kept unchanged, the extraction ratio of polysaccharide first increased and then dropped with increasing dosage ratio of cellulose. As the dosage ratio of cellulose increased from 0.3 to 0.5%, the extraction ratio was found to increase gradually and reached a maximum of 65.25 mg/g at a dosage ratio of cellulose of 0.5%. However, as the dosage ratio of cellulose further increased, less polysaccharide was extracted from Ginkgo mesosperm. Therefore, the dosage ratio of cellulose was set at 0.4, 0.5 and 0.6% for further orthogonal optimization experiment.

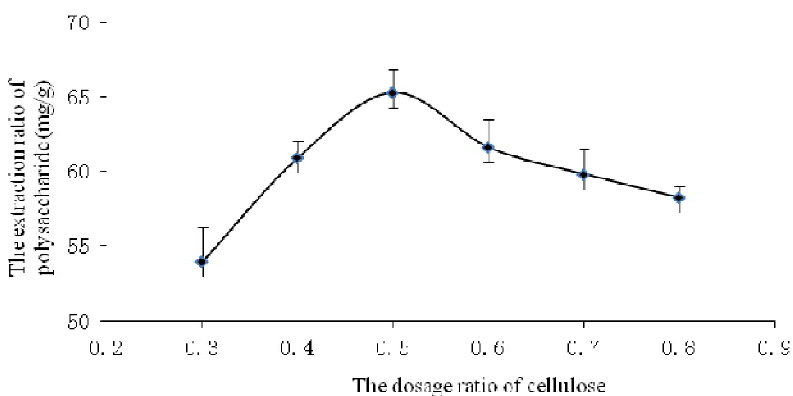


Fig. 3. Effect of the dosage ratio of cellulose on the extraction ratio of polysaccharide from Ginkgo mesosperm.

Fig. 4 shows that the extraction temperature imposed significant effect on the extraction of polysaccharide. If the solid-liquid ratio, the dosage ratio of cellulose and the extraction time are kept unchanged, the extraction ratio of polysaccharide first increased and then dropped with the increasing temperature. As the temperature rose from 50 to 70ºC, the extraction ratio of polysaccharide increased steadily and reached a maximum of 94.611 mg/g at 7ºC; however, the extraction ratio of polysaccharide began to decline as the extraction temperature further increased. This might be due to the fact that the cellulose may become inactive at higher temperature, thereby affecting the extraction ratio of polysaccharide. Therefore, the extraction temperature was set as 65, 70 and 75ºC for further orthogonal optimization experiment.

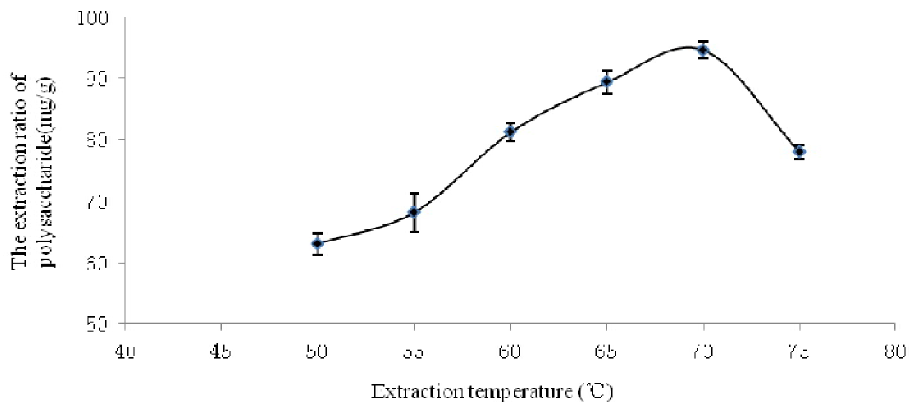


Fig. 4. Effect of extraction temperature on the extraction ratio of polysaccharide from Ginkgo mesosperm.

Table 2 shows the orthogonal experimental results, from which it can be observed that solid-liquid ratio imposed most significant effect on the extraction of polysaccharide from Ginkgo mesosperm, the extraction time, and finally by the dosage ratio of cellulose.

**Table 2. Orthogonal experimental level and results.**

| No. | Extraction temperature (ºC) | Solid-liquid ratio | The dosage ratio of cellulose (%) | Time (min) | The extraction ratio of polysaccharide (mg/g) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 35.883 |
| 2 | 1 | 2 | 2 | 2 | 54.748 |
| 3 | 1 | 3 | 3 | 3 | 89.817 |
| 4 | 2 | 1 | 2 | 3 | 40.027 |
| 5 | 2 | 2 | 3 | 1 | 69.400 |
| 6 | 2 | 3 | 1 | 2 | 99.916 |
| 7 | 3 | 1 | 3 | 2 | 29.942 |
| 8 | 3 | 2 | 1 | 3 | 44.091 |
| 9 | 3 | 3 | 2 | 1 | 94.429 |
| K1 | 180.448 | 105.852 | 179.890 | 197.712 | |
| K2 | 209.343 | 168.239 | 187.204 | 184.606 | |
| K3 | 166.462 | 282.162 | 189.157 | 173.935 | |
| $K_1$ | 60.149 | 35.283 | 59.963 | 65.904 | |
| $K_2$ | 69.781 | 56.080 | 62.401 | 61.535 | |
| $K_3$ | 55.487 | 94.054 | 63.053 | 57.978 | |
| R | 14.294 | 58.771 | 3.09 | 7.926 | |

According to variance analysis results (Table 3), the extraction temperature, the solid-liquid ratio and the dosage ratio of cellulose all significantly affected the extraction of polysaccharide ($p < 0.05$), while the extraction time had no significant effect on the extraction of polysaccharide from Ginkgo mesosperm ($p > 0.05$).

**Table 3. Variance analysis results.**

| Sources of variance | III-type sum of squares | Degree of freedom | Mean square | F | p |
|---|---|---|---|---|---|
| Extraction temperature | 206.407 | 2 | 103.203 | 7.642 | 0.005 |
| Solid-liquid ratio | 15156.862 | 2 | 7578.431 | 561.162 | 0 |
| Dosage ratio of cellulose | 203.685 | 2 | 101.842 | 7.541 | 0.005 |
| Extraction time | 43.482 | 2 | 21.741 | 1.61 | 0.231 |

By analyzing the calculated range results, it may be said that the extraction of polysaccharide from Ginkgo mesosperm can reach a theoretical maximum when the extraction temperature, solid-liquid ratio, extraction time and the dosage ratio of cellulose were set as 70°C, 1 : 10, 80 min and 0.5%, respectively. Since this conditional combination did not appear in the present orthogonal experiment, validation experiment was conducted in accordance with the current extraction technology. After validation, the extracting amount of polysaccharide from Ginkgo mesosperm under this condition was 103.041 mg/g, which was higher than the results under different conditions in the orthogonal experiment.

An enormous number of wastes such as testa, pinecones, bagasse, pericarp and cake are produced in harvesting and utilization of agricultural products, which causes both environmental pollutions and great waste of resources. In recent years, comprehensive processing and utilization of agricultural wastes have aroused great attention from scholars, among which the extraction of polysaccharide is an important direction and has been successfully applied in the processing of oil-tea cakes, rape-seed cakes, pinecones and pericarp (Chen *et al*. 2012, Guo *et al*. 2014). During the harvesting process, testa not only caused great pollution but also brought about huge water of resources. Currently, scientists have extensively investigated the extraction of polysaccharide from the episperm of Ginkgo while neglecting the extraction of polysaccharide from mesosperm. This study focused only on the extraction of polysaccharide from Ginkgo mesosperm using aqueous enzymatic method. Initially, single-factor experiments were performed for investigating the effect of a single factor (namely, extraction temperature, extraction time, the dosage ratio of cellulose and solid-liquid ratio) on the extraction ratio of polysaccharide. Based on the optimal single-factor condition, orthogonal optimization experiment was conducted for determining the optimal combinational condition (an extraction temperature of 70°C, an extraction time of 80 min, a solid-liquid ratio of 1:10 and a dosage ratio of cellulose of 0.5%). Under that optimal condition, the extracting amount of polysaccharide can reach up to 103.041 mg/g. Previous experimental results demonstrated that orthogonal experiment can significantly improve the extraction ratio of polysaccharide from Ginkgo mesosperm, thereby greatly saving energy and enhancing efficiency. The present experimental results can provide theoretical foundations for the development and utilization of Ginkgo mesosperm.

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