Optimization of insoluble dietary fiber preparation technology from *Rosa roxburghii Tratt* pomace by enzyme method

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Abstract. In order to avoid *Rosa roxburghii Tratt* residue wasted. The *Rosa roxburghii Tratt* insoluble dietary fiber was extracted by enzyme method, and the temperature, time, pH and enzyme amount were analyzed by amylase, glucoamylase and protease through single factor and orthogonal experiment. The results showed that the best process of enzymatic extraction of IDF from *Rosa roxburghii Tratt* residue was as follows: the enzymatic hydrolysis 40 ℃ and 70 min, pH 6.0, and the amount of amylase 10 mL; the enzymatic hydrolysis 40 ℃ and 30 min, pH 6.0, and the amount of protease 4 mL; the enzymatic hydrolysis 50 ℃ and 50 min, pH 5.0, and the amount of glucoamylase 8 mL; The average extraction rate of the IDF of *Rosa roxburghii Tratt* residue was 92.41±1.99%, it was dark yellow and could be used as a food ingredient. The experimental method is simple and convenient. It provides a new way for the comprehensive utilization of *Rosa roxburghii Tratt*, and lays a foundation for the development and research of *Rosa roxburghii Tratt*.

1. Introduction
Dietary fiber is a natural, extracted or synthesized carbohydrate polymer in plants, which can not be directly digested and absorbed in the small intestine, but can be fermented and decomposed by intestinal microorganisms in the large intestine [1]. It includes cellulose, hemicellulose, pectin, inulin and other monomer components of dietary fiber [2]. Dietary fiber has attracted more and more attention because of its functions of preventing obesity, preventing diabetes and inhibiting harmful bacteria, and its development and utilization has also been widely recognized [3,4]. Although the research and development of dietary fiber in China started relatively late, the sources of dietary fiber production materials in China are very broad. The residues of various processed foods and by-products of fruit peels can be exploited and utilized in a large number. At present, this part of the fruit and vegetable residue is not only used as animal feed, but also directly discarded, resulting in environmental pollution and waste of resources. *Rosa roxburghii Tratt* is rich in resources and has a wide range of dietary fibers. Among them, insoluble dietary fibers (IDF) have a close structure, which can promote intestinal peristalsis [5,6]. Because of its strong water absorption and reduce the retention time of excreta in the intestine [2], *Rosa roxburghii Tratt* also plays an important role in controlling human body weight [7]. In recent years, many scholars have made many explorations and attempts on the comprehensive utilization of *Rosa roxburghii Tratt*, but the comprehensive utilization and development of its skin residue still lacks a systematic strategy and matching with the development of production and marketing. The extraction methods of dietary fiber include enzymatic method [8], chemical method [9], ultrasound-assisted method [10] and fermentation method [11,12]. The enzymatic method has mild reaction conditions, high purity and less by-products [13,14]. Therefore, the residue of *Rosa roxburghii Tratt* produced in Longli of Guizhou province was used as raw material to degrade other components of the residue by amylase, protease and glucoamylase, so as to extract...
IDF, improve the economic value of *Rosa roxburghii Tratt* residue, and provide experimental basis for the selection of dietary fiber raw materials.

2. Materials and Methods

The *Rosa roxburghii Tratt* residue was collected in the Longli county (Guizhou province, China) in September 2016, which after *Rosa roxburghii Tratt* juice was taken to microwave vacuum, drying, and stored at -20℃ until it was used for the purpose of experiment.

2.1 Extraction process of IDF from *Rosa roxburghii Tratt* residue

*Rosa roxburghii Tratt* pomace→drying and sieving→weigh→add buffer→adjust pH→enzymatic hydrolysis of alpha-amylase (starch decomposition)→boiling water bath inactivation of enzymes→adjust pH→enzymatic hydrolysis of proteins→boiling water bath inactivation of enzymes→adjust pH→glucoamylase enzymatic hydrolysis→boiling water bath→filter→wash→drying→crushing→IDF.

2.2 Enzymatic hydrolysis of protease

The dietary fiber was extracted from *Rosa roxburghii Tratt* pomace by adding 1.0 g pomace into 40 mL tris buffer solution. The effects of different amount of 200 U/mL protease (2, 4, 6, 8, 10 mL), pH value (5, 6, 7, 8, 9), enzymatic hydrolysis temperature (40, 50, 55, 60, 65℃) and time (30, 40, 50, 60, 70 min) on the extraction rate of IDF from *Rosa roxburghii* pomace were studied under the same conditions.

On the basis of single factor experimental data, L₉(₃⁴) orthogonal experiment was carried out with temperature, pH, enzyme addition, time factor and corresponding level. The optimum enzymatic hydrolysis parameters of protease were determined by analyzing the experimental results.

2.3 Enzymatic hydrolysis of glucoamylase

The dietary fiber was extracted from *Rosa roxburghii Tratt* pomace by adding 1.0 g pomace into 40 mL tris buffer solution. The effects of different amount of 200 U/mL glucoamylase (2, 4, 6, 8, 10 mL), pH value (4, 5, 6, 7, 8), enzymatic hydrolysis temperature (40, 50, 55, 60, 65℃) and time (30, 40, 50, 60, 70 min) on the extraction rate of IDF from *Rosa roxburghii* pomace were studied under the same conditions.

On the basis of single factor experimental data, L₉(₃⁴) orthogonal experiment was carried out with temperature, pH, enzyme addition, time factor and corresponding level. The optimum enzymatic hydrolysis parameters of glucoamylase were determined by analyzing the experimental results.

2.4 Enzymatic hydrolysis of alpha-amylase

The dietary fiber was extracted from *Rosa roxburghii Tratt* pomace by adding 1.0 g pomace into 40 mL tris buffer solution. The effects of different amount of 200 U/mL alpha-amylase (2, 4, 6, 8, 10 mL), pH value (5, 6, 7, 8, 9), enzymatic hydrolysis temperature (40, 50, 55, 60, 65℃) and time (30, 40, 50, 60, 70 min) on the extraction rate of IDF from *Rosa roxburghii* pomace were studied under the same conditions.

On the basis of single factor experimental data, L₉(₃⁴) orthogonal experiment was carried out with temperature, pH, enzyme addition, time factor and corresponding level. The optimum enzymatic hydrolysis parameters of alpha-amylase were determined by analyzing the experimental results.

2.5 Yield

IDF yield (%)=IDF dry matter quality (g)/ *Rosa roxburghii Tratt* pomace dry matter quality (g)*100%
2.6 Statistical analysis
Values are expressed as mean +/- standard deviation. Statistical analyses were performed by one-way analysis of variance with the Duncan’s multiple range test and correlation of the data (SPSS 24.0, Illinois, USA). P values of less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1 Effect of protease on yield of IDF from Rosa roxburghii Tratt pomace

3.1.1 Single factor experiments
The effect of the protease on the yield of IDF in *Rosa roxburghii Tratt* pomace was shown in Fig.1. With the increase of the amount of protease, the yield of IDF gradually increased, and the highest yield was obtained at the addition of 4 mL (p<0.05). With the continuous increase of enzyme dosage, the yield of IDF in *Rosa roxburghii Tratt* residue did not continue to increase and maintained a steady trend. This may be due to the reduction of substrate and accumulation of products, resulting in no further enhancement of enzymatic reaction [14].

Fig.1(B) shows that with the change of enzymatic hydrolysis temperature, the yield of IDF from *Rosa roxburghii Tratt* pomace fluctuates. The yield of IDF from *Rosa roxburghii Tratt* pomace is significantly higher than that of other groups (p<0.05) when the enzymatic hydrolysis temperature is 40, 55 and 60 °C. When the enzymatic hydrolysis temperature higher than 60 °C, the enzyme activity of protease is affected by the excessive temperature of enzymatic hydrolysis, and the structure of hemicellulose and cellulose in dietary fiber may change [15, 16]. It decreased significantly (p<0.05). Therefore, the three groups with the highest IDF yield (40, 55 and 60 °C) were selected as the three levels of the next orthogonal test.

The effect of enzymatic hydrolysis time on IDF of *Rosa roxburghii Tratt* pomace is shown in Fig.1(C). The prolongation of enzymatic reaction time promotes the enzymatic reaction of protease [17,18]. Therefore, the yield of IDF increases at 30 and 40 min. With the prolongation of reaction time, the decomposable substances in *Rosa roxburghii Tratt* pomace decrease gradually, and the reaction products increase and accumulate, which may inhibit the enzymatic reaction [17], leading to the yield of IDF from slag was significantly lower than that of other groups, and then the extraction rate tended to increase, which may be due to the change of soluble matter structure [18,19]. Therefore, the hydrolysis time of protease was 30, 40 and 70 min for orthogonal experiment.

As shown in Fig.1(D), the yield of IDF increased with the increase of pH value. The yield of IDF was the highest at pH 6, but the effect of pH value on the yield of IDF was not significant (p>0.05), indicating that pH had little effect on the activity of protease in the experimental range.

![Figure 1. Effect of protease on yield of IDF](image-url)
3.1.2 Orthogonal experiment  According to the four factors of enzymatic hydrolysis temperature, time, pH value and adding amount of protease, and the three optimal levels determined by single factor experiments of these factors, the orthogonal table $L_9(3^4)$ was selected to optimize. The specific design is shown in Table 1. The orthogonal experimental results showed that the order of protease affecting the yield of IDF from *Rosa roxburghii Tratt* pomace was pH>enzyme dosage>temperature>time, and the optimum technological conditions for extracting IDF from *Rosa roxburghii Tratt* pomace by this method were D2A1B1C1: the amount of protease was 4 mL, the temperature was 40 ℃, the pH was 6, and the time was 30 min. In the orthogonal experiment, there is no coincidence test with the best process, and then verify the experiment according to this process, the yield of IDF is (89.41± 0.97)%.

| number | A (concentration/mL) | B (temperature/℃) | C (time/min) | D (pH) | yield (%)    |
|--------|----------------------|-------------------|--------------|--------|-------------|
| 1      | 1(4)                 | 1 (40)            | 1 (30)       | 1 (5)  | 88.18       |
| 2      | 1                    | 2 (55)            | 2 (40)       | 2 (6)  | 86.13       |
| 3      | 1                    | 3 (60)            | 3 (70)       | 3(7)   | 83.38       |
| 4      | 2(6)                 | 1                 | 2            | 3      | 84.54       |
| 5      | 2                    | 2                 | 3            | 1      | 84.47       |
| 6      | 2                    | 3                 | 1            | 2      | 86.58       |
| 7      | 3(8)                 | 1                 | 3            | 2      | 85.67       |
| 8      | 3                    | 2                 | 1            | 3      | 82.09       |
| 9      | 3                    | 3                 | 2            | 1      | 82.06       |
| K1     | 85.897               | 86.130            | 85.617       | 85.170 |
| K2     | 85.197               | 84.230            | 84.510       | 86.127 |
| K3     | 83.540               | 84.273            | 84.507       | 83.337 |
| R      | 2.357                | 1.900             | 1.110        | 2.790  |

3.2 Effect of glucoamylase on yield of IDF from *Rosa roxburghii Tratt* pomace

3.2.1 Single factor experiments  As shown in Fig.2(A), the enzymatic hydrolysate of starch was further degraded to monosaccharide with the addition of glucoamylase, so that the yield of IDF from *Rosa roxburghii Tratt* pomace increased first, and the yield was higher than that of other groups when the amount of glucoamylase was 8 mL. However, the amount of enzyme was increased, the yield could not be improved. It was possible that the cellulose and hemicellulose components in *Rosa roxburghii Tratt* residue could be degraded into oligosaccharides or monosaccharides such as glycoside bond and hydrogen bond, which could not be easily precipitated by ethanol, resulting in the gradual decrease of the extraction rate of IDF.

In the range of 40-60 ℃, the change of IDF yield was not significant with the increase of enzymatic hydrolysis temperature, but the highest yield was obtained at 65 ℃, as shown in Fig.2(B). This is inconsistent with the changes of protease and amylase affected by temperature, indicating that the temperature range of enzymatic reaction of glucoamylase is wide, and is similar to the optimum
temperature of enzymatic hydrolysis of dietary fiber extracted from potato dregs by glucoamylase [14]. In summary, the orthogonal experiments were carried out at 40, 50 and 65 ℃.

The IDF yield of Rosa roxburghii Tratt pomace increased slowly with the prolongation of enzymatic hydrolysis time, suggesting that the increase of enzymatic reaction time would make Rosa roxburghii Tratt pomace participate in enzymatic hydrolysis better. However, the yield of IDF decreased rapidly after 60 minutes (p<0.05), which may be due to the decrease of Rosa roxburghii Tratt residue and the accumulation of dietary fiber with the increase of reaction time. Therefore, the negative feedback inhibition effect of reaction products increased gradually [20], resulting in the decrease of reaction efficiency, so that the yield no longer increased with the increase of enzymatic hydrolysis reaction time. In view of this, three higher levels of 40, 50 and 60 min were selected for the next orthogonal experiment.

The effect of pH value on the yield of IDF is shown in Fig.2(D). The yield of IDF from Rosa roxburghii Tratt pomace shows increasing trend. It shows that the enzyme has good catalytic activity in the optimum pH range. With the increase of pH value, the enzymatic reaction is stronger [14,17]. However, when the pH value exceeds 6, the enzymatic activity decreases and the enzymatic hydrolysis is inhibited [14,17], which eventually reduced the yield of IDF from 86.1% to 79.2% (p<0.05).

3.2.2 Orthogonal experiment  The orthogonal experiment was carried out according to the three optimum levels determined by the single factor experiment. The L_9 (3^4) orthogonal table was selected to optimize the preparation process. The specific design is shown in Table 2. The orthogonal experimental results show that the order of influencing the yield of IDF from Rosa roxburghii Tratt pomace is time<pH<enzyme dosage<temperature; and the optimum technological conditions for extracting IDF from Rosa roxburghii Tratt pomace by this method are B2A3D1C2: 8 mL protease dosage enzymatic hydrolysis for 50 min at 50 ℃ and pH 5. The yield of IDF was 91.26±1.51%, which was higher than other groups.

![Figure 2. Effect of glucoamylase on yield of IDF](image)

| number | A (concentration/mL) | B (temperature/°C) | C (time/min) | D (pH) | yield (%) |
|--------|---------------------|--------------------|--------------|--------|-----------|
| 1      | 1(4)                | 1 (40)             | 1 (40)       | 1 (5)  | 86.29     |
| 2      | 1                   | 2 (50)             | 2 (50)       | 2 (6)  | 88.36     |
| 3      | 1                   | 3 (65)             | 3 (60)       | 3 (7)  | 89.18     |
| 4      | 2(6)                | 1                  | 2            | 3      | 84.32     |
| 5      | 2                   | 2                  | 3            | 1      | 88.22     |
3.3 Effect of amylase on yield of IDF from Rosa roxburghii Tratt pomace

3.3.1 Single factor experiments

The yield of IDF was increased after filtration, because the starch contained in Rosa roxburghii Tratt pomace was hydrolyzed gradually with the addition of amylase [16,19]. The starch in Rosa roxburghii Tratt pomace was gradually decomposed with the increase of the amount of amylase, and the yield of IDF was gradually increased. When the amount of amylase was 6 mL, the yield was significantly higher than that of other groups (p<0.05), and nearly 15% higher than that of 2 mL enzyme solution. As starch was hydrolyzed completely gradually, the increase of amylase could not further improve the yield of IDF [14].

As shown in Fig.3(B), amylase had little effect on IDF yield of Rosa roxburghii Tratt pomace in the temperature range of 40-60°C (p>0.05), In this range, the rate of water molecule movement accelerated, which promoted the enzymatic reaction [14,16], and the yield of IDF was higher (p>0.05), which was significantly higher than that at 65°C (p<0.05). It is suggested that the structure of amylase may change and the activity of amylase may begin to decrease when the temperature of enzymatic hydrolysis exceeds 60 ℃, thus affecting the enzymatic hydrolysis ability of amylase. The starch hydrolysis in pomace samples is not complete, or some IDF are degraded into soluble substances, resulting in a decrease in the yield of amylase [13,21].

Fig.3(C) shows that the contact time between amylase and Rosa roxburghii Tratt residue is increased with the prolongation of enzymatic hydrolysis time, which makes the starch in raw materials fully enzymatic hydrolysis, thus increasing the yield of IDF (p<0.05). However, when heated for 60 min, there was a downward trend (p<0.05). The enzymatic hydrolysis time continued to increase, which may lead to the conversion of some soluble dietary fibers into IDF [14,16], resulting in a significant increase in the yield at 70 minutes (p<0.05).

The effect of pH value on the yield of IDF from Rosa roxburghii Tratt pomace is shown in Fig.3(D). pH has a significant effect on enzyme activity and reaction environment. With the increase of pH, the yield of IDF increased gradually (p<0.05). The yield of IDF at pH 6,was significantly higher than that of other groups (p < 0.05). This indicated that this condition was the optimum pH value for amylase. When pH was higher than 6, the activity of amylase was inhibited, or some IDF increased its solubility under the strong hydrolysis of alkaline environment, so the yield decreased (p<0.05).
3.3.2 Orthogonal experiment

After preliminary analysis of the factors affecting the yield of IDF from *Rosa roxburghii Tratt* pomace by alpha-amylase, the orthogonal experiment of $L_9(3^4)$ was carried out on the basis of single factor experiment with pH, enzymatic hydrolysis temperature, enzymatic dosage and time. The level table was shown in Table 3. The results of orthogonal experiment showed that the order of influence on the yield of IDF was as follows: enzyme dosage<pH<time<temperature, and enzymatic hydrolysis time was the main reason for the treatment of IDF by amylase. According to range analysis, the optimum extraction technology of alpha-amylase was determined as $B_3C_1D_1A_3$: temperature 40 ℃, pH 6, enzyme dosage 10 mL and time 70 min. According to the above conditions, the optimum extraction rate of IDF from alpha-amylase was 92.41±1.99%.

| number | A (concentration/mL) | B (temperature/℃) | C (time/min) | D (pH) | yield (%) |
|--------|----------------------|--------------------|--------------|--------|-----------|
| 1      | 1(6)                 | 1(40)              | 1(40)        | 1(6)   | 88.12     |
| 2      | 1                    | 2(50)              | 2(50)        | 2(7)   | 86.04     |
| 3      | 1                    | 3(40)              | 3(70)        | 3(8)   | 87.74     |
| 4      | 2(8)                 | 1                  | 2            | 3      | 87.08     |
| 5      | 2                    | 2                  | 3            | 1      | 86.84     |
| 6      | 2                    | 3                  | 1            | 2      | 86.56     |
| 7      | 3(10)                | 1                  | 3            | 2      | 91.08     |
| 8      | 3                    | 2                  | 1            | 3      | 80.47     |
| 9      | 3                    | 3                  | 2            | 1      | 90.52     |
| $K_1$  | 87.300               | 89.093             | 85.033       | 88.493 |
| $K_2$  | 86.827               | 84.433             | 87.880       | 88.227 |
| $K_3$  | 87.673               | 88.273             | 88.887       | 85.080 |
| $R$    | 0.846                | 4.660              | 3.854        | 3.413  |
The results showed that the yield of IDF from *Rosa roxburghii Tratt* residue treated with amylase, protease and glucoamylase was much higher than that of purple sweet potato dietary fiber [22], coconut dietary fiber [20], taro [16] and potato [14] dietary fiber prepared by enzymatic method. Among them, for amylase and glucoamylase, enzymatic hydrolysis temperature has the greatest impact on the yield of IDF; for protease, pH condition has the greatest impact on the yield, which may be due to the different enzyme activity or its own characteristics.

4. Conclusion

With the increasing pursuit of dietary health, the IDF was extracted from *Rosa roxburghii Tratt* residue by enzymatic method in this paper. Starch, protein and sugar were removed from the fruit residue samples by amylase, protease and glucoamylase. Single factor and orthogonal experiments were carried out to optimize the process of the three enzymes, including the amount of the three enzymes added, enzymatic hydrolysis temperature, time and pH; adding buffer solution to *Rosa roxburghii Tratt* residue, the pH value was 6, 2000 U amylase, 40 °C constant temperature water bath 70 min, then 800 U protease enzymatic hydrolysis 30 min at 40 °C and pH 6.0, adding glucoamylase 1600 U, pH 5.0, 50°C enzymolysis 50 min, solid-liquid separation after enzyme deactivation, the yield of IDF was up to 92.41±1.99% after drying. This method not only has a high yield, but also has mild conditions. The obtained IDF can be directly applied to food raw materials. It can also alleviate the pressure of environmental pollution caused by *Rosa roxburghii Tratt* and lay a foundation for the comprehensive utilization and development of *Rosa roxburghii Tratt* pomace.

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