Study on Extraction Method of rosemary antioxidant

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Abstract. The antioxidant components in rosemary were extracted by organic solvent method. The optimum extraction conditions of rosemary antioxidant were studied by changing the solvent, ratio of material to liquid, extraction time and environment. Considering the factors of energy consumption, efficiency, cost and environmental protection, the optimum extraction condition of rosemary antioxidant is using ethanol as solvent and hot reflux for 2 hours, and the ratio of liquid to material is 10:1.

1. Introduction
Rosemary extract is safe, efficient, heat-resistant and broad-spectrum antioxidant, which has become one of the key research and development of food antioxidants in recent years. In addition, the functions of rosemary essential oil components in medical and health care, such as anti-inflammatory, anti-tumor, anti-cancer, treatment of cardiovascular diseases, liver protection and enzymatic reduction and so on, have also attracted great attention of researchers at home and abroad. Domestic literature shows that the main antioxidant components of rosemary are terpenoids, phenols and acids. Rosemary essential oil, which is separated from rosemary, is a traditional European fragrance. In addition, rosemary essential oil also has the efficacy of sterilization, insecticidal and anti-inflammatory. It is widely used in perfume, bath liquid, cosmetics, shampoo, air freshener, ant repellent and other daily chemicals. As spices, rosemary has been widely used in food industry. A lot of in-depth studies have been done on its extracts in food preservation, color protection, oil stabilization and meat flavor stabilization [1-10]. The results show that rosemary antioxidants have incomparable superiority. In this paper, antioxidants were extracted from rosemary and their main components were analyzed and identified by various means.

2. Materials and experimental instruments
Rosemary dried leaves: Guangxi Baise Huagui Biological Co., Ltd. Fourier transform infrared spectrometer: WQF-410, produced by Beijing Second Optical Instrument Factory. Mass Spectrometer: QP-2010 A, Shimadzu Corporation, Japan. High performance liquid chromatography: Waters 600, Waters Company, USA. Liquid Chromatography-Mass Spectrometer: LCQ DECA XP, Thermo Company, USA.
3. Extraction of rosemary antioxidant
The extraction methods of antioxidants from rosemary mainly include organic solvent extraction [11], and supercritical fluid extraction [12-14], among which organic solvent extraction is the most commonly used method. According to the different extraction conditions, organic solvent method can be divided into heating stirring reflux method, cold immersion method and ultrasonic assisted extraction method [15]. In this experiment, the organic solvent method was used to study the optimum extraction conditions of rosemary antioxidants by changing the solvent, material-liquid ratio, extraction time, environment and other factors.

The extraction steps are as follows: the leaves and stems of rosemary are dried, crushed to 80 meshes, extracted with organic solvents under various conditions, filtered, concentrated, washed, dried, dissolved, activated carbon adsorption and decolorization, and dried at 60 °C to produce rosemary extract.

3.1. Effect of the solvents on the yield of antioxidants
After drying and crushing, 50 g rosemary was extracted by heating and refluxing with 500 ml organic solvent for 2 hours. The solvents used were ether, ethyl acetate, chloroform, ethanol, petroleum ether, acetone and methanol. The quality and yield of the extracts were shown in Table 1.

| Solvent       | aether | ethyl acetate | chloroform | ethanol | petroleum ether | acetone | methanol |
|---------------|--------|---------------|------------|---------|----------------|---------|----------|
| Quality of extracts / g | 6.71   | 7.71          | 7.33       | 8.22    | 6.22           | 7.15    | 9.40     |
| Yield / %     | 13.42  | 15.42         | 14.66      | 16.44   | 12.44          | 14.30   | 18.80    |

The yields of the products extracted by different solvents are different. The yield of methanol extract is the highest, but the residual solvent is toxic. The yield of ethanol extract is second only to that of methanol, but the residual solvent is non-toxic, so ethanol is chosen as the extraction solvent.

3.2. Effect of the ratio of material to liquid on the yield of antioxidants
50 g rosemary raw material was heated and refluxed in ethanol for 2 hours. The amount of ethanol was 250 ml, 500 ml, 625 ml, 750 ml, 875 ml and 1000 ml, respectively. The quality and yield of the extract were shown in Table 2.

| Solvent dosage / mL | 250 | 500  | 625  | 750  | 875  | 1000 |
|---------------------|-----|------|------|------|------|------|
| Quality of extracts / g | 5.33| 8.22 | 8.30 | 8.51 | 8.40 | 8.50 |
| Yield / %           | 10.66| 16.44| 16.60| 17.02| 16.80| 17.00|

Table 2 shows that the yield of antioxidants decreases with the increase of solvent dosage. When the solvent is increased to 500 ml and then the amount of solvent is increased, the yield of the extract will not be significantly increased. Considering the cost factor, the optimum liquid-to-material ratio is 10:1.
3.3. Effect of extraction time on the yield of antioxidants

The extraction time of 50 g rosemary was 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h, respectively. The quality and yield of the extract were shown in table 3.

| Time / h | 0.5 | 1   | 2   | 3   | 4   | 5   | 6   |
|----------|-----|-----|-----|-----|-----|-----|-----|
| Quality of extracts / g | 6.00 | 7.31 | 8.22 | 8.10 | 8.40 | 8.61 | 8.60 |
| Yield / %    | 12.00 | 14.62 | 16.44 | 16.20 | 16.80 | 17.22 | 17.20 |

The yield of antioxidants increased significantly with the increase of extraction time after 0.5h, 1h and 2h, but there was no significant difference between the yield after 2H and that after 2h, so 2H was the best extraction time.

3.4. Cold-soaked extraction

The above extraction experiments of rosemary antioxidants are carried out under the condition of heating reflux, which requires a certain amount of energy. The cold soaking method is to immerse the rosemary raw materials directly in the solvent without heating.

50 g rosemary was soaked in 500 ml ethanol for 1 d, 3 d, 5 d and 7 d, respectively. The quality and yield of the extract were shown in table 4.

| Time / d | 0.5 | 1   | 3   | 5   | 7   |
|----------|-----|-----|-----|-----|-----|
| Quality of extracts / g | 6.62 | 8.02 | 8.00 | 7.78 | 7.79 |
| Yield / %    | 13.24 | 16.04 | 16.00 | 15.56 | 15.58 |

It can be seen that the yield of rosemary antioxidant has approached the level of heating reflux for 2 hours after 1 day of cold immersion. In the case of low time requirement, this energy-free extraction method can be chosen. The yield of antioxidants increased significantly with the increase of time at 0.5 d and 1 d, and then changed slightly. Therefore, the optimum extraction time for extracting rosemary antioxidants by cold immersion with ethanol as solvent was 1 d.

3.5. Ultrasonic wave extraction

50 g rosemary was immersed in 500 ml ethanol and extracted with ultrasound. The extraction time was 0.5 h, 1 h, 1.5 h and 2 h, respectively. The quality and yield of the extract were shown in table 5.

| Time / d | 0.5 | 1   | 1.5 | 2   |
|----------|-----|-----|-----|-----|
| Quality of extracts / g | 2.81 | 1.64 | 3.71 | 4.80 |
| Yield / %    | 5.62 | 3.28 | 7.42 | 9.60 |

The yield of antioxidants increased with the increase of extraction time within 2 hours of ultrasound-assisted extraction, but there was a big gap between the yield under this condition and that under the condition of hot reflux.

4. Conclusion

Considering the factors of energy consumption, efficiency, cost and environmental protection, the optimum extraction condition of rosemary antioxidant is ethanol as solvent and hot reflux for 2 hours, and the ratio of liquid to material is 10:1.

According to the market survey feedback in China, it is estimated that more than 2,000 tons of natural antioxidants are needed in China every day in the fields of oil, canned food, meat products, instant
noodles, fried food, pastries and beer. The development and commercial promotion of rosemary essential oil as an antioxidant can scavenge free radicals and prevent oil from oxidative deterioration. It can be predicted that the high economic value and wide application of rosemary antioxidant will promote the further development of rosemary industry.

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