
The Advances of Preparation and purification of sulforaphane

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Abstract

Sulforaphane can be obtained from cruciferous vegetables as one of the products of enzymatic or acid hydrolysis of glucoraphanin, which has been identified the best anticancer activity. The sulforaphane's preparation and purification methods were summarized, their development orientation was pointed out, and theoretical and technical references were provided in aspects of the sulforaphane's preparation, analysis and large-scale industrialization production.

Keywords

Sulforaphane; Preparation; Purification.

1. Introduction

Sulforaphane can be obtained from cruciferous vegetables as one of the products of enzymatic or acid hydrolysis of glucoraphanin, which has been identified the best anticancer activity. It was first discovered in radishes[1]. It has been proved that broccoli is rich in anticancer substances, which can reduce the formation of mouse breast tumors and prevent the activity of carcinogenic substances [2]. In early 1997, a study of cancer-fighting vegetables by British scientists showed that broccoli and Brussels sprouts were rich in glucose-isothiocyanates. Sulforaphane has been isolated from glucose isothiocyanate, and the DNA material with sulforaphane growth gene has been isolated from broccoli. The implantation of this material into various cabbage and radish will help people resist cancer cells and reduce the risk of cancer [3]. The Brassica Chemooprotection laboratory at Johns Hopkins university school of medicine in the United States has found that broccoli contains high levels of isothiocyanates, which activate the body's own anticancer substance, Phase Two Enzymes[4]. This enzyme neutralizes suspected carcinogens and prevents them from damaging genetic factors in healthy cells. Japan's agricultural research institute also indicated that isothiocyanate can prevent the growth of melanin cancer cells [5]. Sulforaphane is by far the most anticancer active isothiocyanate found in vegetables, and its anticancer effect has been fully proved in the breast cancer of rats [6].

Although there have been some studies on the transformation of glucosinolates into isothiocyanates, there is a lack of research on the regulatory conditions and methods for large-scale extraction and separation of isothiocyanates. So we strengthen to this kind of natural, has a strong effect of preventing and treating cancer substances - sulforaphane research, provide a scientific basis for further development of anti-cancer drugs for the future, in line with the international demand for drugs, green, environmental protection, non-toxic, thus to better development and utilization of green vegetables broccoli to provide scientific theory basis.

2. Preparation method of sulforaphane

The preparation methods of sulforaphane have become the focus of many scientists. The existing methods include chemical synthesis, enzymatic extraction, chemical synthesis and enzymatic extraction.

2.1 Chemical synthesis

In the 1990s, James et al. synthesized sulforaphane by chiral synthesis in stereochemistry. The synthesis of sulforaphane from the perspective of stereochemistry is simple but difficult to control the reaction conditions. A sulforaphane chemical synthesis method is based on potassium *o*-phenyldimethylamine, which is reacted with 1, 4-dibromobutane to form *n*-alkyl *o*-phenyldimethylamine. Alkyl amine is obtained by hydrazine hydrolysis, and sulforaphane is then reacted with sulforaphane to form sulforaphane with a total yield of about 16% [7]. Whitesell et al. [8] proposed a chiral synthesis method, using chiral adjuvant *trans*-2-phenylcyclohexanol as raw material, and obtained *r*-sulforaphane through multiple reactions. The reaction process of chemical synthesis is easy to control, and easy to produce in large quantities. However, generally using toxic reagents as raw materials, it is easy to cause environmental pollution, and the synthetic product is racemate. However, the chiral synthesis method has many steps, complicated process and low yield.

2.2 Half a synthesis

Half a synthetic method of the structure of the radish thioglycoside analogues, such as glucoraphenin, glucoerucin as raw materials, such as turnip glucosinolates is obtained by biological or chemical process, and then hydrolyzed sulforaphane. The content of glucoraphenin in radish seeds is one more double bond than that of sulforaphenin. Therefore, glucoraphenin is taken as the raw material, and hydrogenated with platinum oxide, carbon palladium and other catalysts under high temperature and pressure, and then sulforaphane is hydrolyzed. But the method has a lower conversion rate. Half a synthesis has the advantage of cheap raw materials, radish seeds are rich in glucoraphenin, arugula seeds are rich in glucoerucin, these seeds are tap cheaply. Therefore, if the conversion efficiency can be improved, this method is expected to have a good application prospect. But the disadvantages of this approach are sulforaphane is extracted after enzyme sterilization, and exogenous glucosinolate is added to the sulforaphane to hydrolyze the sulforaphane [9].

2.3 Enzymatic extraction

Sulforaphane precursors (glucosulforaphane glucosinolate) were extracted from cruciferous vegetables or their seeds by enzymolysis and obtained by hydrolysis. There are 4 kinds of glucosulforaphane hydrolysis products, the most common are sulforaphane (SF) and sulforaphane (SFN). The hydrolysis conditions (pH value, temperature, degree of hydration, metal ions (Fe^{2+} , Fe^{3+}), mercaptan, etc.) have a great influence on the proportion of hydrolysis products. Therefore, the control of hydrolysis conditions in this method is of vital importance. Due to the complex process of sulforaphane enzymatic hydrolysis and many influencing factors, the enzymatic hydrolysis and extraction process optimization of sulforaphane are also an important research content. In the reports on the optimization of enzymatic hydrolysis and extraction conditions, the optimization methods are mostly used to investigate the interaction of solid-liquid ratio and hydrolytic pH by orthogonal or response surface experiments on the basis of single factor. The process parameters reported in these studies vary greatly, which indicates that the enzymatic hydrolysis and extraction of sulforaphane is a complex process with many influencing factors.

Ultrasound and microwave assisted extraction have been widely used in the extraction of plant active ingredients. Compared with the traditional solvent extraction method, ultrasonic assisted extraction method has fewer solvents and shorter extraction time, while microwave has the characteristics of strong penetration, high selectivity and high heating efficiency. Tanongkankit et al [10]. studied the influence of different solvent, microwave power and extraction time on the yield of sulforaphane using cabbage as raw material, and optimized the extraction process. Pongmalai et al [11]. also showed that microwave extraction of sulforaphane has higher energy efficiency. Brions-labarca et al [12]. extracted sulforaphane from the seed of Chilean papaya (*Vasconcellea pubescens*), and compared the high-pressure extraction method, ultrasonic assisted extraction method and traditional extraction method. The results showed that the high-pressure extraction method had the highest extraction efficiency, followed by the ultrasonic assisted extraction method, which had the lowest extraction

rate. The experiment of tang bin et al. showed that the extraction efficiency was improved by ultrasonic extraction.

2.4 Chemical synthesis and enzyme extraction were combined

The combination of chemical synthesis and enzymatic hydrolysis was obtained from seeds of arugula, and glucosulforaphane was prepared by selective oxidation reaction using glucosyltrieradiate as raw material, and sulforaphane was obtained by hydrolysis. This method overcomes the difficulty of separating glucosulforaphane from glucosulforate isothiocyanate extracted by HPLC through enzymatic hydrolysis, and has high reaction efficiency, which is expected to realize the industrialization of sulforaphane production.

3. Purification of sulforaphane

The purification methods of sulforaphane include macroporous resin adsorption, silica gel column chromatography, reverse high performance liquid chromatography and high-speed countercurrent chromatography.

Sulforaphane has good adsorption performance on the macroporous resin. Therefore, the macroporous resin adsorption is an ideal method for sulforaphane purification. Literature [13] reported the purification of sulforaphane by the method of macroporous adsorption resin. Through the adsorption of SP850 resin, the alcohol solution was eluted, and the purity of sulforaphane was more than 85%. High purity sulforaphane can be obtained by reverse high performance liquid chromatography. After defatting, enzymolysis and extraction of broccoli seeds, sulforaphane can be isolated and purified by reversed-phase chromatographic column. The mobile phase is acetonitrile/water, methanol/water or acetone/water. Forward silica gel column chromatography can also be used to purify sulforaphane. Liang et al[14]. used silica gel as fixed phase and trichloromethane/methanol (95:5) as mobile phase to obtain more than 90% purity of sulforaphane.

4. Conclusion

Sulforaphane was prepared mainly by extracting glucosulforaphane from cruciferous plants and seeds. The extract was extracted with organic solvent after enzymolysis or acid hydrolysis, and then extracted with fixed phase. Broccoli relative to the seed prices cheaper, and the higher levels of sulforaphane, looking for a kind of nontoxic and cheap solvent for extraction solvent, and assisted other physical means from broccoli extract sulforaphane, avoid the use of toxic organic solvents extraction steps, extract residue can be used as feed, can save resources, reduce costs, protect the environment at the same time. It is in line with the international requirements of green, environment-friendly and non-toxic drug development, and can better develop and utilize the green vegetable broccoli.

Application of new purification process. Molecular distillation, for example, has been widely used as a special and developing distillation separation technique, which is particularly suitable for the separation of high boiling point, heat sensitivity and easy oxide. Sulforaphane is easy to be oxidized and degraded in separation and purification, and its boiling point is high. If this technology is applied to the separation and purification of sulforaphane, not only the yield of the product can be improved, but also the quality of the product can be guaranteed to some extent.

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