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Note

Novel high pressure extraction technology

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Abstract

This note describes a novel application of the food processing technology, known as high pressure processing (HPP), to the extraction of essential components of herbs. Herbal extracts may be used as drugs, as well as ingredients in food and cosmetics. We have run some pilot studies in our laboratories to demonstrate the applicability of high pressure extraction.

Keywords: High pressure extraction; Essential components; *Epimedium*

The natural extracts of herbs can be used as drugs, as well as ingredients (including flavor, color, etc.), in food and cosmetics. They may be used in place of synthetic chemicals, and have caught the attention of biologists, chemists, pharmaceutists, doctors, nutritionists, etc. There are many extraction methods, such as soxhlet, heat reflux, boiling, and distilling methods, which are traditional (Conghou, 1987), and ultrasonic, microwave, and supercritical fluid (CO_2 and gases, named pressure extraction or high pressure extraction (HPE) by some) technologies, which are newly developed (Bruneton, 1999). Here, we show a novel method: high pressure extraction. HPE is neither high pressure homogenization (also named high pressure extraction or high pressure cell breakage) nor the high pressure supercritical fluid; it is the same as the high pressure processing of food (HPP) (Kinetics of Microbial Inactivation). By HPE, the essential components (EC) of herbs can be extracted in the shortest time; there are few impurities in the extracting solution of HPE, and the single component of high purity can be easily obtained. Strong, weak, and non polar compounds can all be extracted by using different solvents. Saving energy and safety are also advantages of HPE. HPE could be used as a tool for drug discovery, allowing chemical reactions to occur under more suitable conditions.

High pressure processing of food is a method of processing food products with no or minimal heat treatment. Pre-packaged or post-aseptically packaged food can be treated under elevated pressure up to 880 MPa (130,000 psi). Some highly acidic foods have been pasteurized by the process, and the pressure-processed foods are reported to have better flavor, texture, nutrition retention, and color compared to thermally processed foods. Because the conditions are isostatic, it is possible to process non-pumpable foods, such as meat products, by HPP.

Such a process may also be of use in the pharmaceutical industry for the extraction of EC from herbs. It is named high pressure extraction by us.

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Table 1
Comparison of different processing methods for the extraction of essential components (EC) from herbs

EC	Process	Condition	Duration	AR (%)	Indication
Icariin from *Epimedium*	HPE	70% EtOH, 600 MPa	5 min	10	PDE/5 (libido supplement)
	Reflux	70% EtOH	2 h × 3	6.9	
	Ultrasonic	70% EtOH	1 h	7.2	
Polyphenols from green tea	HPE	80%EtOH, 380 Mpa	10 min	28	Antioxidant/clearance of free radicals
	Boiling water	Boiling water	45 min	24	
Flavonoids from *Propolis*	HPE	90% EtOH, 500 MPa	1 min	5.2	Antibacterial activity
	EtOH Leaching	EtOH leaching	1.5 h × 2	4.7	
Baicalin from *Scutellaria*	HPE	MeOH, 200 MPa, 0 MPa, 600 MPa	5 min each	3.1	NNRTI (HIV)
	Reflux	MeOH	2.5 h	1.2	

We have run some pilot studies in our laboratories to demonstrate the applicability of HPE. Some results are provided in Table 1.

Here, high pressure means cold, isostatic ultra high hydraulic pressure (CIP). The process of HPE involves mixing the raw herb with a solvent, treating the mixture with CIP, and filtering to remove the solid particles. The solution can be concentrated, dried, or further purified to obtain a single component.

Many organic compounds in herbs are heat-sensitive (Bruneton, 1999), i.e. if heated, they will be denatured, loose biologic activity, or change into another compound. The extraction of these components is very difficult to achieve with traditional methods, but can easily be achieved through HPE. With HPE, operating at room temperature, the denaturation affected by the heat is avoided; so the heat-sensitive components can be extracted easily, particularly volatile oils, which are very useful in drugs and cosmetics.

There are many components in herbs, and the polarity of herbal components is different. Thus, the extracting solvent has to be matched to the polarity of the component being extracted, as is done in traditional methods. The supercritical CO_2 is a lipophilic solvent (Stahl, 1980). Water, hydrophilic, and lipophilic organic solvents of different concentrations can all be used in HPE. Thus, HPE can be used to extract strong, weak, and non-polar molecules by using different solvents. Glucides, coumarins, lignans, quinines, flavonoids, terpenes, tannins, triterpenoids, cardiac heterosides, glycosides and aglycones, alkaloids, etc. can all be extracted using HPE.

In general, the solubility of most natural compounds is very high under high pressure (von Rohr, 1996) or solvent of higher density (Ziqiang, 2000). The pressure range used at HPE is 100–1000 MPa. As a comparison, the highest pressure of the high pressure supercritical fluid is about 100 MPa, the pressure of supercritical CO_2 is normally (Ziqiang, 2000) about 10 MPa, and other extracting methods mentioned above is only 0 MPa, i.e. the pressure of HPE is the highest in all extracting technologies. Thus, HPE can extract more of the EC and the achievable ratio (AR, mass of EC in solution/mass of raw herb) of HPE is the largest. The comparison of different processing methods for the extraction of EC from herbs is shown in Table 1.

The protein will be denatured (Kinetics of Microbial Inactivation) and the membranes of the cell will be destroyed (Bennett, 1998); under high pressure, more solvent will enter the inner of cells and more EC will be extracted out of cells more easily. But the cells aren't broken into clastics, which is the result of most of the extracting technologies, particularly high pressure homogenization.

There are only a few impurities in the extracting solution of HPE if the operating condition is chosen judiciously. For example, under different operating conditions of HPE, the color of the extract of *Epimedium* is different as shown in Fig. 1. The color of icariin solution is yellow; the solution of darker color contains more impurities. The operating conditions of HPE can be controlled to reduce impurities, and the solution of HPE can be purified easily to get a single compound of high purity. First, an *Epimedium*

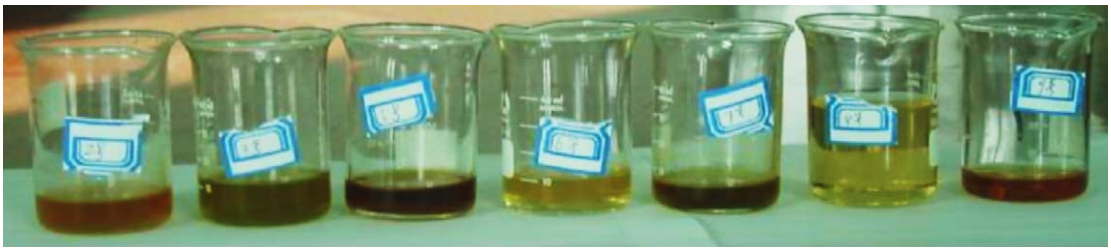

Fig. 1. The color of the extraction solution obtained by HPE. The conditions of HPE, from left to right: (1) 200 MPa holding 5 min, 0 MPa holding 5 min, 600 MPa holding 5 min, solvent is 50% ethanol; (2) pressure and holding time are same as 1, solvent is 70% ethanol; (3) 400 MPa holding 5 min, solvent is 70% ethanol; (4) 400 MPa holding 10 min, solvent is water; (5) 600 MPa holding 5 min, 0 MPa holding 5 min, 600 MPa holding 5 min, solvent is 70% ethanol; (6) 600 MPa holding 5 min, solvent is water; (7) 600 MPa holding 10 min, solvent is 50% ethanol.

extract is obtained from 50% ethanol/H_2O by holding for 5 min at 350 MPa by the HPE process. Then, the pure icariin can be isolated from the yellow extract obtained from the *Epimedium* by prep LC with no pre-treatment or by liquid–liquid extraction.

According to mass transfer theory (Huixing, 2002), the rate of mass transfer = pressure/resistance of mass transfer. Based on phase behavior theory (Sadus, 1992), the dissolution is faster at higher pressure. The differential pressure between the inner and the exterior of the cell is very large under HPE conditions. Under this large differential pressure, the solvent will permeate very fast through the broken membranes into cells, and the mass transfer rate of solute or the rate of dissolution is very large. This leads to a very short extracting time with HPE, compared to normal and supercritical fluid extraction technologies. A lot of researchers claim that if the spore of *Ganoderma lucidum* isn't broken, the polysaccharide cannot be extracted. By HPE, AR of polysaccharide of unbroken *G. lucidum* spore is 1.433%, which is same as broken. The extracting time of HPE, i.e. holding time as mentioned above, is only 5–20 min; however, the boiling or refluxing method takes 1–3 h, and supercritical CO_2 extraction takes about 3 h (Ziqiang, 2000) or more.

Limited by the energy level, weak bonds, such as the hydrogen bond, the electrostatic bond, the Van der waals bond, and the hydrophobic bond, can be broken by high pressure, but the covalent bond can't be broken, so the small molecule will not change under high pressure (Kinetics of Microbial Inactivation). Most of extraction by HPE is for small molecules, i.e. EC will not change during HPE under normal temperature. However, many reactions may be obtained under high pressure, such as stereoisomerization, pericyclic reaction, anionic reaction, synthesis (le Noble, 1988), hydrogenation (von Rohr, 1996), inorganic and bioinorganic reaction (Winter, 1998). These and other reactions may also occur with HPE, i.e. EC, which we are trying to extract, may be changed. Some changes may be harmful for the drug, and for ingredients of food and cosmetics. But, we can surely find the beneficial changes. Perhaps, it will be an important method for new drug discovery.

For example, the *Scutellaria* is treated by heat reflux (methanol, 1 h); the solution (SBZ) contain two compounds, baicalin and baicalein, and their molal weights are 445 and 269, respectively. When *Scutellaria* is treated by HPE (methanol, 200 MPa holding 5 min; 0 MPa holding 5 min; 600 MPa holding 5 min), the solution (SBM) only contains one compound, and its molal weight is 272. Compare the biological activity of SBZ and SBM by the multiplication test of lymphocyte; both can inhibit the lymphocyte transformation of the mouse which is induced by ConA, 2.5 μg/ml; SBM, 31.25 μg/ml; and SBZ, 125 μg/ml. So, we know that some reactions occur and the biological activity increases after HPE treatment.

Under different pressures, holding times, solvents and concentrations, aids etc., the denaturation of protein and the destruction of membranes are different (Kinetics of Microbial Inactivation; Bennett, 1998). This means that we can choose the composition of extraction solution, i.e. extract different activity component and/or control impurity content. For example, when the concentration of ethanol is less than 50%, the pressure is less than 400 Mpa,

and the holding time is less than 10 min, there is no chlorophyll in the *Epimedium* solution after HPE treatment. This means that the chloroplast membrane hasn't been destroyed. To extract tea polyphenols from green tea by HPE when the solvent is water, the AR increases by 50% if 0.5% ethanol is used as an aid.

There is no power required during pressure holding and decompression of HPE. The power has to be supplied only when increasing pressure, which is the same as HPP. Therefore, in addition to shorter extracting time, HPE also helps to save a lot of energy. Because the actuating medium is liquid but not gas, HPE is safer than gas medium at high pressure.

To summarize, HPE is a very useful tool for the extraction of natural products. It has economic and scientific values. Yet what we know about HPE is far from enough; many physical, chemical, biological and medical theories, and engineering problems remain to be explored. However, more examples remain to be explored using this technique.

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