Research Progress of Deep Eutectic Solvents in Extraction of Plant Flavonoids

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Abstract As a new type of green solvents, deep eutectic solvents (DESs) have the advantages of strong extraction ability, designability, simple preparation, low price, recoverability and biodegradation, and show great application potential in the field of plant flavonoid extraction. In this paper, the definition, classification and preparation methods of DESs were introduced. The effects of DES composition, molar ratio of DES components, water content of DES systems, liquid-material ratio, extraction temperature, extraction time and extraction auxiliary techniques on the extraction yield of plant flavonoids were expounded. The recycling methods of DESs were summarized. Existing problems of DESs in the field of plant flavonoids extraction were pointed out, and further research direction and trend were analyzed and prospected.

Key words Deep eutectic solvent; Plant flavonoid; Extraction; Assistive technique; Regeneration

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Flavonoids are widely distributed in the plant kingdom. Up to now, more than 2 000 kinds have been found. Most of them are distributed in roots, stems, leaves and other parts of plants in the form of glycosides, and a few of them exist in free form[1-2]. The basic nucleus of flavonoids is a structure formed by connecting two phenyl groups through a three carbon chain, that is, they have a C6-C3-C6 basic skeleton. Common compounds include flavonoids and flavonols, dihydroflavones, isoflavones, chalcones, flavanols, anthocyanins, *etc.* (the general structural formulas of flavonoids and anthocyanins are shown in Fig. 1 and Fig. 2)[1,3]. Modern pharmacological research shows that flavonoids have antioxidant, antibacterial, anti-inflammatory, anti-aging, hypoglycemic, antitumor, blood vessel blockage prevention and heart protection effects, and can be widely used in food (as natural food additives, they can prevent oil oxidation, reduce nitrite consumption and inhibit acrylamide generation, *etc.*), medical care, daily chemical products, aquaculture and other fields[4-5].

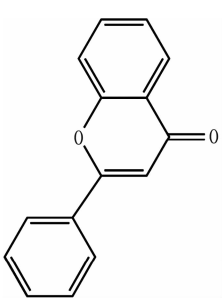

Fig. 1 Flavonoids and flavonol compounds

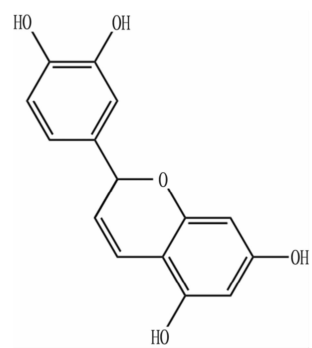

Fig. 2 Anthocyanin compounds

There are many methods for extracting flavonoids, and the traditional methods are mainly water extraction and organic solvent extraction. Lin[6] and Zhao *et al.* [7] found that the water solubility of flavonoid glycosides in hot water is increased, and samples can be boiled in water and then filtered and concentrated to obtain target products. However, due to the high polarity of water, protein, sugar and other components are easy to be extracted, and there are many problems such as impurities, difficulty in separation and purification, and easy rot and deterioration of extracts during storage stored[4]. However, the organic solvent extraction method often uses ethanol, acetone, ethyl acetate, ether and other substances. The study by He *et al.* [8] shows that flavonoids have high polarity and are easily soluble in ethanol, and compared with water extraction, the extraction amount of flavonoids is significantly increased, and the method is simple and convenient to operate, and can give consideration to both nutritional value and pharmacological function requirements and can realize effective extraction of flavonoids. However, organic reagents are flammable, volatile, non-degradable, toxic, solvent-consuming, and remain easily. Long-term use will not only pollute the environment, but also limit the application of flavonoid extracts in medicine, food and other fields due to solvent residues[3,5]. In order to overcome the limitations of organic extraction, scientists have developed new extraction

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techniques, such as microwave-assisted extraction, ultrasonic-assisted extraction, supercritical extraction, homogenate-assisted cavitation extraction and so on. BAGADE *et al.* [9] found that microwave magnetic field can significantly improve the extraction efficiency of flavonoids. Li[10] thinks that although the extraction rate of microwave-assisted water extraction is lower than that of ethanol thermal reflux method, it is environmentally friendly, harmless to human body and has good reproducibility. However, the microwave extraction method has the attenuation problem when the microwave penetrates the substance, which affects its application[11]. Ultrasonic wave has cavitation effect. Under the action of ultrasonic wave, plant intracellular fluid produces strong shock wave and micro-jet, which lead to multiple secondary effects, such as crushing, emulsification, diffusion and strong mechanical oscillation, and accelerates the dissolution of plant flavonoids. Ou[5] thinks that ultrasonic technique is excellent in the extraction rate of flavonoids, low costs, simple operation and reduction of energy consumption, but its potential noise interference needs to be improved. Supercritical extraction and homogenate-assisted negative pressure cavitation extraction can achieve the characteristics of no solvent residue and efficient extraction of flavonoids while maintaining their activity, but they face problems such as complex equipment, high energy consumption, and slow processing speed[12-13].

With the proposing of the concept of green extraction technology, the development of green solvents has become one of the hot spots in green chemistry research. In 2003, deep eutectic solvents (DESs) were first proposed by Professor Abbott of the University of Leicester, England. They show outstanding characteristics of environmental protection, simple preparation, low price, biodegradability, *etc.*, and have the advantages of good compatibility, recoverability, high extraction capacity, designability, *etc.* They have attracted extensive attention from researchers in many fields such as organic synthesis, extraction separation, environmental analysis, and functional material synthesis[14-15]. In the extraction of natural products, DESs have been applied to the extraction of active components such as polysaccharides, alkaloids and polyphenols[16]. DESs have great application potential in the extraction of flavonoids because of its adjustable polarity and viscosity.

In this paper, the definition, classification and preparation methods of DESs were introduced, and the research progress of DESs in flavonoids extraction was expounded, and the separation and recovery methods of DESs were summarized. Furthermore, existing problems of DESs in flavonoids extraction were pointed out, and the application of DESs in flavonoid extraction and separation was prospected, aiming to provide reference for the development and application of new flavonoids extraction solvents.

Definition, Classification and Preparation Methods of DESs

Definition

A DES is a binary or ternary eutectic mixture composed of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) in a certain molar ratio[17]. Hydrogen bonding force, van der Waals force and π-π force will be formed between different compounds that make up DESs, which makes lattice energy decrease, and ions can move freely at room temperature to become liquid, resulting in eutectic phenomenon with lower melting point, that is, the freezing point of compounds is significantly lower than the melting points of pure substances of various components[18-20]. Abbott found that the mixture of choline chloride (melting point: 302 ℃) and urea (melting point: 133 ℃) with a molar ratio of 1: 2 can form a liquid with a melting point of 12 ℃ after heating and cooling to room temperature[14]. Such deep eutectic solvents have low volatility, thermal stability, good solubility and designability of ionic liquids, as well as the good characteristics of easy preparation, recoverability and biodegradability, so they are a new type of green solvents.

Classification of DESs

The design of a DES system should first select a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). According to the types of ligands, DESs can be divided into four types[19,21]. ① The first is quaternary ammonium salts as the HBA, amide, carboxylic acid and polyol as the HBD, such as choline chloride and ethylene glycol. This type of deep eutectic solvents is the most commonly used, and their principle is that the melting point decreases due to the hydrogen bond interaction between anions in quaternary ammonium salts and hydrogen bond donors. Abbott *et al.* [14] studied the effects of HBA structure, HBD structure and molar ratio on the melting point of DESs, and found that the stronger the ability of HBAs to accept hydrogen bonds and HBDs to donate hydrogen bonds, the lower the melting point of DESs. ② The second is quaternary ammonium salts and aqueous metal chlorides, such as choline chloride and $CrCl_3 \cdot 6H_2O$. The formation of this type of metal salt solvents is mainly due to the charge delocalization formed by covalent bond between the anion X- of metal salt and ligand Y, which leads to the decrease of melting point[22]. ③ The third is quaternary ammonium salts and anhydrous metal chlorides, such as choline chloride and AgCl. As in ②, charge delocalization is mainly formed by anion X- of metal salt and ligand Y. ④ The fourth is metal halides ($AlCl_3$, $ZnCl_2$, $FeCl_3$, *etc.*) and HBDs (urea, acetamide, ethylene glycol, *etc.*), such as $ZnCl_2$ and urea. For this type of solvents, the principle is that the metal cation $[mx_{n1}]^+$ and metal anion $[mx_{n3}]^-$ formed by asymmetric splitting of metal halides and hydrogen bond donors reacts to form a complex, which decreases the charge density, and then reduces the coulomb force between anion and cation, thus achieving eutectic[23].

Preparation method of DESs

Different preparation methods have differences in preparation temperature, moisture content and pressure, which will affect the physical and chemical properties and stability of DESs. Even the DESs obtained by a given preparation method still has performance differences in practical application. Therefore, when preparing DESs, it is necessary to choose a suitable method to obtain an expected effect[15,24]. Several commonly used preparation methods of DESs are introduced below.

Heating method Heating is the most commonly used and simple method to prepare DESs. When the components of DESs are dry compounds and have good thermal stability, this method can be adopted. Various components are mixed, added in a closed container and heated at a certain temperature until a uniform and transparent solution or colloid is formed. Xin *et al.* [25] mixed choline chloride (ChCl) and trehalose at 80 ℃ and heated the mixture, and finally obtained transparent DESs.

Grinding method When choline chloride and carboxylic acid are used as components of DESs, if the system temperature is higher than 100 ℃, Cl in choline chloride and -COOH in carboxylic acid may react to generate impurities, which will affect the stability and solvent effect of DESs. Florindo *et al.* [26] put various components into a mortar and ground them, and the components reacted with each other under continuous mixing to form DESs. In the process of preparing DESs by grinding, there are some problems such as moisture absorption and unstable solvent properties, so the method has certain limitations[27].

Rotary evaporation method or vacuum evaporation method Rotary evaporation preparation (or vacuum evaporation) is different from the above two methods, as it needs the participation of a third component (such as water and methanol) except the HBA and HBD. During preparation, all HBA and HBD are dissolved in the solvent, and then added in a rotary evaporator or vacuum evaporation equipment, and most of the solvent is removed by evaporation until the weight is constant, finally obtaining a uniform and transparent DES liquid[28]. Wikene *et al.* [29] dissolved the components in warm water and evaporated them in a rotary evaporator at 45 ℃ for 15 min to prepare NADESs.

Freeze drying method The Freeze-drying method is to dissolve various components of DESs in ultra-pure water, and then mix all aqueous solutions evenly, and then, water was removed by vacuum freeze-drying to obtain a uniform and viscous transparent colloid[30]. This method cannot completely remove all water molecules in DESs, and water molecules may react with DESs components. Moreover, it has high requirements for equipment and takes a long time for treatment, so it is rarely used. Gutierrez *et al.* [31] mixed choline chloride and urea aqueous solution evenly, froze the prepared aqueous solution, and then dried it in vacuum, finally obtaining transparent DESs.

Research on the Application of DESs in Extraction of Flavonoids

The ability of DESs to extract flavonoids depends on the composition of DESs (types of HBA and HBD, their molar ratio), physical and chemical properties (polarity, solubility, viscosity, surface tension, *etc.*) and the interaction between DESs and the target, while the water content and temperature of DESs will directly affect the physical and chemical properties of DESs. In addition, the ratio of material to liquid, extraction time, auxiliary means for extraction and so on will also affect the extraction yield of flavonoids.

Composition of DESs

Different types of components lead to different physical and chemical properties of DES systems, such as polarity and solubility, and their extraction ability to bioactive substances will be very different. Hydrophilic DESs show better effect in extracting flavonoids with strong polarity. At present, choline chloride (ChCl) is the most commonly used HBA, followed by betaine and L-pro, and sodium acetate, ammonium acetate, D-maltose and lactic acid are occasionally reported. More commonly used HBDs are alcohols (ethylene glycol, propylene glycol, glycerol, *etc.*), organic acids (malic acid, lactic acid, oxalic acid, *etc.*), amides and urea[3,32–35]. Yao *et al.* [36] used natural deep eutectic solvents to extract flavonoids from *Ginkgo biloba* leaves, and studied and screened DES systems with choline chloride as the HBA and urea, lactic acid, citric acid, malonic acid, oxalic acid and glycerol as the HBD. The results showed that the best DES was the combination of choline chloride and glycerol. Sun *et al.* [37] used choline chloride as the HBA, glycerol, 1,3- butanediol, 1,4-butanediol, ethylene glycol and glucose as the HBD. The optimal DES system consisted of choline chloride and 1,4-butanediol, and the extraction rate of total flavonoids from Flos Chrysanthemi Indici was 53.85 mg/g. According to the results, it was speculated that the position of hydroxyl group in polyols would also affect the extraction effect. Liu *et al.* [38] used choline chloride, sodium acetate and ammonium acetate as the HBA, acetic acid, lactic acid and ethylene glycol as the HBD, and screened out the optimal DES system for total flavonoids in Fructus Aurantii Immaturus as the combination of choline chloride and acetic acid.

Molar ratio of DES components

The composition of DESs and the molar ratio between HBA and HBD determine whether eutectic systems can be formed and whether the systems can exist stably. CIARDI *et al.* [39] found that the number of groups provided by HBDs is the key to the formation of DESs. In NADESs composed of lactic acid and glycine, when the molar ratio of HBD∶HBA is equal to or smaller than 3, no liquid can be formed at room temperature, and only when HBD∶HBA is greater than or equal to 5 can a stable eutectic system be formed. However, if HBD∶HBA is too large, the relative concentration of HBA in the system may be insufficient, which will affect the formation of hydrogen bond network. Wang *et al.* [33] used choline chloride/lactic acid system to extract total flavonoids from *Dichondra micrantha* Urban, and investigated the effects of HBA∶HBD molar ratios of 1∶1, 1∶2, 1∶3 and 1∶4 on the extraction rate of total flavonoids. The results showed that with the increase of molar ratio, the extraction rate first increased and then decreased, and the extraction rate was the highest when HBA∶HBD was 1∶2. Yang *et al.* [40] used betaine/L-proline/butanediol system to extract total flavonoids from *Tribulus terrestris*. With the increase of HBD, the extraction rate first increased and then decreased. When the ratio of the two was 1∶2, the extraction rate reached a maximum, which might be because of the largest number of hydrogen bonds formed at this time. When the HBD continued to increase, too-much competition of HBD made the hydrogen bond network unstable, which led to a decrease in the extraction rate of flavonoids. However, Sun *et al.* [37] studied with choline chloride and different polyols, and found that with the same HBD, DESs with a molar ratio of 1∶3, had lower viscosity and surface tension than DESs with a molar ratio of 1∶2, which made it easier for solvents to penetrate into cells and promoted the dissolution of total flavonoids from Flos Chrysanthemi Indici.

System water content

There are a large number of hydrogen bond networks, van der Waals forces and electrostatic interactions among the components of DESs, which greatly reduce the fluidity of systems and show high viscosity, while high viscosity will hinder the contact between solvents and target compounds and reduce the extraction yield. The viscosity and polarity of DESs can be adjusted by adding a certain proportion of water. Water is polar molecules, which can form hydrogen bonds with components in DESs and weaken the interaction between components. The increase of water content can reduce the viscosity of DESs, enhance the surface tension of DESs, facilitate the formation of hydrogen bonds between DESs and target compounds, and improve the solubility of target compounds[41]. However, excessive water will also destroy the hydrogen bond between components of DESs, resulting in the reduction of the extraction rate of target compounds. Dai *et al.* [42] found that when the water content was above 50%, the interaction between HBA and HBD would weaken or even disappear with the dilution of water, so an appropriate water content is very important in practical application.

Ratio of material to liquid

The ratio of liquid to material plays an important role in the conduction of target compounds. Increasing the amount of solvent is beneficial to increasing the contact area of liquid and material, make the material fully infiltrated, promote the diffusion of flavonoids from the inside of cells to DES systems, and improve the yield. However, if the liquid material is too much, it will cause waste, and increase the dissolution of non-target components and the difficulty of purification. In addition, if ultrasonic-assisted extraction is adopted, the ultrasonic effect received by the liquid material per unit volume may be reduced, which may lead to a decrease in the total yield of flavonoids. Therefore, it is necessary to control an appropriate liquid-material ratio. Li *et al.* [43] extracted flavonoids from *Scutellaria baicalensis* Georgi with the ratios of liquid to material (ml/g) at 10: 1, 20: 1, 30: 1 and 40: 1. The results showed that the total yield of flavonoids increased first and then decreased with the increase of the ratio of liquid to material, and the yield of flavonoids was the highest when the ratio of liquid to material was 20: 1. Wei[44] studied the extraction of flavonoids from *Morinda citrifolia* residue with the ratios of liquid to material (ml/g) at 10: 1, 20: 1, 30: 1, 40: 1 and 50: 1. The results showed that the extraction rate increased from 10: 1 to 20: 1, and was stable from 20: 1 to 50: 1, showing no significant difference. It might be because the dissolution amount of flavonoids was relatively fixed, and too high liquid-material ratio had little effect on the extraction rate of flavonoids, but it would waste a lot of heat and increase the cost during concentration.

Extraction temperature

High temperature will weaken the interaction of ionic bonds in DES systems and reduce the viscosity of the systems. In addition, with the increase of temperature, the movement of DES molecules is enhanced, which can promote the penetration of DESs into tissues and cells and improve the probability of contact between DESs and target compounds. These two effects can promote the dissolution of flavonoids in DESs and improve the extraction rate of flavonoids, but attention should be paid to the destruction of flavonoid structure by high temperature. Kong *et al.* [45] extracted total flavonoids from apple leaves at 40, 50, 60, 70, 80 and 90 ℃ by ultrasound. It was found that the extraction rate of total flavonoids was positively correlated with the temperature in the range less than 70 ℃, and reached a maximum at 70 ℃. With the continuous increase of temperature, the extraction rate of flavonoids began to decline, which might be caused by the destruction of flavonoids structure by high temperature in the later period. This trend is similar to that obtained by Sun[37], Liu[38] and Wei[44], but the optimum extraction temperature may be slightly different.

Extraction time

Ni *et al.* [46] used DESs to extract total flavonoids from green tea, and found that with the extension of extraction time, the extraction of total flavonoids increased significantly at first, and then tended to balance. When the extraction time was 60 min, the extraction process was basically completed, and the extraction rate no longer increased. However, the research results of Xu *et al.* [47] are different. Under the conditions of extraction time of 40, 50, 60, 70 and 80 min, the total flavonoids in broccoli buds first rose and then fell, and the extraction rate was the highest at 70 min.

In the study of extracting plant flavonoids by DESs, many scholars will also use other technical methods to improve the extraction rate, among which ultrasonic-assisted extraction is the most used. The research shows that ultrasonic time has a significant effect on the extraction rate of plant flavonoids. With the increase of ultrasonic time, the extraction rate gradually increases, but after reaching a certain value, it will show a downward trend. The reason may be that in the early stage, a longer ultrasonic time leads to greater capacity generated by the ultrasound and stronger cavitation effect formed in the solution, and the extraction rate of flavonoids is thus higher. However, long-term ultrasonic treatment may destroy the structure of flavonoids and cause the degradation of target components, but the extraction amount will decrease. Zhang *et al.* [48] investigated the effects of ultrasonic time of 10, 20, 30, 40, 50 and 60 min on the extraction rate of flavonoids from jujube residue, and found that the extraction rate was the highest at 40 min. Du *et al.* [49] took the ultrasonic time of 5, 10, 15, 20 and 25 min as variables, and the results showed that the extraction rate of flavonoids increased in the range of 5 – 15 min, and then decreased. It was analyzed that the reason for the decrease might be the degradation of flavonoids due to the dual effects of ultrasonic radiation and thermal effect, and the change of the internal chemical structure of flavonoids due to long-term extraction, which led to the unstable interaction between DESs and samples and affected the extraction effect.

Auxiliary means for extraction

On the basis of using DES systems to extract plant flavonoids instead of traditional organic solvents, researchers usually use ultrasonic technique and microwave-assisted extraction technique to optimize the extraction process to improve the extraction rate of target compounds.

Ultrasonic-assisted extraction technique Ultrasonic-assisted extraction of flavonoids is widely used. It is considered that ultrasonic wave can increase the movement frequency and speed of substances, increase the penetration of solvents, improve the dissolution speed and times of target compounds and promote the release of target compounds. In the extraction of plant flavonoids, air bubbles are formed inside DESs under the action of ultrasonic waves, and when the air bubbles can't absorb the applied mechanical waves, they will burst and release a lot of heat and shear force. These factors act together on the target products, prompting flavonoids to dissolve more in DES systems[50]. Du *et al.* [49] used ultrasound and DESs to extract flavonoids from *Osmanthus fragrans*. Under optimized process conditions, the extraction rate of flavonoids was 8.93 mg/g higher than that of traditional alcohol extraction. Fu *et al.* [51] used ultrasonic-assisted DES systems to extract flavonoids and polyphenols from pine needles of *Pinus massoniana*, and found that under optimized conditions, the yield of flavonoids was 10.377%, which was 8% higher than that of ethanol extraction under the same conditions, and the amount of solvent used was reduced by 5 times.

Microwave-assisted extraction technique Microwave-assisted extraction technique can also be applied to the extraction of plant flavonoids by DESs. Sun *et al.* [52] used microwave-assisted DESs to extract total flavonoids from chickpeas. Under optimized extraction conditions of response surface methodology, the extraction rate of total flavonoids was 2.49 mg/g, which was as high as 90.55% compared with the value obtained by analysis and detection (the content of total flavonoids in chickpeas was about 2.75 mg/g), while the extraction rate of total flavonoids from chickpeas was only 75.23% by traditional ethanol extraction (70% ethanol). Li *et al.* [53] thought that microwave-assisted extraction has the advantages of high efficiency, rapidity and solvent saving, and DESs have strong absorption and conversion ability to microwave, and the combination of the two can realize green and efficient extraction of total flavonoids from leaves of *Coix lachryma-jobi*.

Compared with ultrasonic-assisted extraction, the application of microwave-assisted extraction in flavonoid extraction is relatively less, which may be because microwave extraction has the characteristics of selective heating. Generally, solutes and solvents with greater polarity have greater absorption of microwave, and the temperature rises faster, and the extraction speed is thus faster. DESs have a wide polarity range. If microwave-assisted extraction of plant flavonoids is adopted, it is necessary to investigate the polarity of DES systems to obtain a better extraction yield[54].

Enzyme-assisted extraction technique Choosing suitable biological enzymes can destroy the cell wall structure dominated by cellulose and pectin connected between cells, reduce the mass transfer resistance of extraction, make flavonoids in plants fully released[55], and improve the extraction yield of flavonoids by DESs. Wu *et al.* [56] innovatively combined DESs, enzymes and ultrasound to extract total flavonoids from mulberry leaves, and compared them with traditional water extraction, alcohol extraction, ultrasound-water extraction and ultrasound-alcohol extraction. The results showed that ultrasound and enzyme-assisted DES extraction achieved the highest yield, and was more time-saving and environmentally friendly.

Mechanochemical method——ball mill-assisted extraction technique The ball mill method is to use mechanical force to make the sample achieve ultrafine grinding and change chemically with surrounding solid or liquid after grinding, thereby achieving efficient extraction of target compounds. Liu *et al.* [57] established an extraction method of flavonoid glycosides from bamboo leaves based on DESs and ball mill. The method is simple and efficient, and has been improved compared with traditional methods in shortening sample preparation time and protecting environment.

Negative pressure cavitation-assisted extraction technique Negative-pressure cavitation extraction technique is a new extraction technique based on cavitation bubble formation theory and cavitation erosion effect. Its most basic feature is bubble phenomenon. Bubbles can change the hydrodynamic properties of mixtures and significantly affect the mixing, mass transfer and heat transfer of chaotic systems. Negative-pressure cavitation extraction technique has the advantages of high extraction rate, simple equipment, easy operation and low energy consumption, and has been widely used in efficient extraction of active components in medicinal plants, but few studies have been carried out on the extraction of plant flavonoids by combining it with DESs. Pan *et al.* [58] applied this technique to the extraction of tectorigenin and other substances from *Dalbergia odorifera* leaves, and found that the extraction effect of DESs assisted by negative pressure cavitation (the extraction rate of tectorigenin was 1.057 mg/g) was better than that of ultrasonic extraction (1.044 mg/g) and thermal reflux extraction (0.988 mg/g).

Cyclic regeneration of DESs

The recycling of DESs is not only related to the economic cost, but also required by environmental protection, thus playing an important role in its industrial application.

DESs have the advantages of low saturated vapor pressure and high thermal stability. For light component solutes (such as light aromatic hydrocarbons, pyridine, *etc.*), DESs can be separated from solutes by vacuum distillation, thereby achieving recycling of DESs. For heavy component solutes (such as dibenzothiophene, bioactive components, *etc.*), DESs cannot be separated from solutes by distillation or rectification, and but can be recovered by adding a back-extractant or an anti-solvent (such as ether, methyl-tert-butyl ether, water, *etc.*) to precipitate the solutes[17,59]. Cheng *et al.* [59] selected water as an anti-solvent and used the strong interaction between water and DESs to adjust the interaction between DESs and solutes. When the amount of water was small, it promoted the solutes to dissolve into DES systems, while when the amount of water was large, it destroyed the stability of DESs and separated the solutes from DES systems again. This method can complete the cycle process of extraction and back extraction by adjusting the water content in systems, and it is green and environmentally friendly without the participation of organic solvents in the whole process, but it is only suitable for situations where the solutes are hydrophobic components.

It has also been reported that supercritical carbon dioxide extraction, solid phase extraction and adsorption chromatography are used to separate the components to be detected from DESs. Among them, the macroporous resin adsorption method can be used to separate various DES systems from solutes, and it is considered to be a simple and effective method for DES recovery and utilization. Xia *et al.* [35] investigated the adsorption rates of three different types of macroporous resins for total flavonoids extracted from *Prunella vulgaris* by DESs. Under the optimized conditions, AB-8 resin could basically completely adsorb the total flavonoids, and due to the direct adsorption of macroporous resin, DES solvents could be completely recovered and applied to the next cycle of extraction, and the yield of total flavonoids extracted from *P. vulgaris* for the second time could reach 95.23% ($n = 3$). Experiments show that DES solvents are recyclable and can be reused.

Summary and Prospects

DESs, as a new type of extraction solvents, have the advantages of simple preparation, low cost, environmental protection, strong biological affinity, designable system and high recycling rate, and have become a research hotspot in the field of extraction of plant active components, especially in the extraction of flavonoids. Many studies have found that using DESs instead of traditional organic solvents to extract plant flavonoids often leads to higher extraction yield, especially with the assistance of ultrasound, microwave and other techniques.

However, as far as the current research situation is concerned, while recognizing the advantages of DESs, it is also necessary to fully recognize problems existing in the application of flavonoid extraction. ① The design of DES systems is not sufficient, and the HBAs in DESs are limited to choline chloride, betaine and L-pro, *etc.*, and there have been few studies on other hydrogen receptors. Moreover, they are basically binary systems, and few studies have been conducted on ternary or above. ② Most of the research is limited to the investigation of flavonoid extraction rate, and few studies have been conducted on how to separate and purify flavonoids after extraction. ③ DESs are usually assisted by ultrasound, microwave, negative pressure cavitation and enzymolysis in the extraction of flavonoids, but the research simply shows that the extraction rate is high, and the research on mechanism has not been carried out, so it is impossible to clarify the reason for the high extraction rate. ④ DES reagents can be recycled, but the research on how to effectively separate solvents and solutes without affecting the activity of flavonoids and introducing new and environmentally-friendly factors after they are used to extract flavonoids, is very rare.

In the future, aiming at problems of DESs in the field of flavonoid extraction, the research can focus on four aspects. ① It is necessary to continue to develop more types of DESs, expand the screening scope of HBAs and HBDs, deeply study the formation and mechanism of DESs, and directionally design and prepare DESs with stronger ability to extract flavonoids. ② The purity and quality of flavonoids extracted by DESs can be studied, and the quality standard of flavonoids extracted by DESs should be established, and the research on separation and purification needs to be studied, so as to provide basis for the subsequent study on the properties and application of flavonoids. ③ The recycling performance of DES solvents for extracting flavonoids needs to be further investigated, so as to truly realize the efficient utilization of extraction solvents. ④ It is necessary to explore the mechanism of various auxiliary techniques in affecting the yield of flavonoids extracted by DESs, in order to screen and obtain a more perfect "DES + auxiliary technique" extraction process of flavonoids.

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of the village party secretary who is directly responsible for cooperative construction, innovate and implement the new industrial chain development model of "party organization + cooperative + base + farmer + marketing", and effectively build party branches on the industrial chain, so as to enrich the people in the industrial chain and stimulate more people to join cooperatives actively. According to local conditions, the government should guide each township (town) to create 1 – 2 well-run and strongly-driving model cooperatives, and set up a model for the masses to see and learn, so as to achieve the goal of making the masses actively participate in cooperative work through realistic dividends.

Conclusions

Farmers' professional cooperatives are the future development direction of rural production and management system and an important way to promote agricultural scale operation and centralized development. However, in the domestic and international environment of agricultural products market, with the deepening of market economy and the development of globalization, the operation and management mechanism of farmers' professional cooperatives is not perfect enough, and the scale of operation is not large enough, and professional management talents are still lacking. As a result, the market competitiveness is not strong enough.

In order to break the bottleneck of development, farmers' professional cooperatives should not only give full play to their own advantages to overcome their disadvantages, but also seize the opportunity to meet the challenges and constantly improve their comprehensive management level. Meanwhile, the government needs to further increase its support, solve difficulties and problems in the development of farmers' professional cooperatives, and make overall plans to optimize social resources. It is also necessary to cultivate a professional talent team, actively research and develop agricultural products with industrial characteristics, and create brand names with agricultural products. Meanwhile, related managers should constantly innovate marketing models, realize a higher level of agricultural industrialization and a larger scale of operation, and guide farmers' professional cooperatives to develop sustainably in a healthy direction.

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