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Biomass Extraction Methods

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1. Introduction

Biomass represents an extremely valuable potential to obtain new clean energy sources and natural structurally complex bioactive compounds. Renewable energy can be produced from any biological feedstock, that contains appreciable amounts of sugar or materials that can be converted into sugar (e.g. starch or cellulose). Lignocellulose's biomass–dendromass and phytomass is natural based material consisting of complex of heterogenic macromolecules with cell structure (celluloses, hemicelluloses and lignin) as well as numerous organic and inorganic structures with low molecule weight (Sun, 2002).

Long-term economic and environmental concerns have resulted in a great amount of research in the past couple of decades on renewable sources of liquid fuels to replace fossil fuels. Producing of cellulose and alcohol from biomass is important technological process. Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions. Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. The use of biomass with low economic value, the waste from agriculture, forestry and wild flora as sources of clean energy, is a viable way to avoid potential conflicts with the biomass production for food, which represent the main concern of UE regarding the biofuels production from biomass.

The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For the conversion of biomass to fuel, the cellulose and hemicellulose must be broken. The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. The lignocellulosic biomasses need to be treated prior to fuel production to expose

cellulose. In present, there is many different type of pretreatment of lignocelluloses materials. Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion, to alter the structure of cellulosic biomass to make cellulose more accessible. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Then, acids or enzymes can be used to break down the cellulose into its constituent sugars. Enzyme hydrolysis is widely used to break down cellulose into its constituent sugars. Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in efficiency and lowering of costs through further research and development. Cellulose chains can also be broken down into individual glucose sugar molecules by enzymes known as cellulase. Cellulase refers to a class of enzymes produced by fungi, bacteria, and protozoans that catalyze the hydrolysis of cellulose. But, one of the main drawn back of convention chemical methods used in ethanol formation process is degradation of carbohydrates and formation of undesirable by-products, which severely inhibition of ethanol during the fermentation process: furfural, 5-hydroxymethylfurfural, uronic acid, levulinic acid, acetic acid, formic acid, hydroxybenzoic acid, vanillin, phenol, cinnamaldehyde, formaldehyde, and so (Nenkova et.al., 2011). Some inhibitors such as terpene compounds are present in the biomass–dendromass.

Lignin is a complex reticulated phenolic polymer that occurs in xylem of most terrestrial plants and is the second most abundant biopolymer in nature, corresponding to around 30% of the biosphere organic carbon. This macromolecule is one of the biggest wood components and also one of the most important. Even the lignin has a significant role in technology, in the bioethanol production process valuable chemical properties and functions from lignin and hemicelluloses are not fully recovery, the black liquor result from process being using specially for energy recovery. About half of wood components are dissolved into this black liquor. The dissolved organic compounds consist mainly in degraded lignin and also hemicelluloses and cellulose degradation products. Also, phenols derived from biomass are valuable and useful chemicals, due to their pharmacological properties including antiviral inhibitor (anti-HIV). These compounds with good antioxidant activity can be used to preserve food from lipid peroxidation and oxidative damage occurring in living systems (Martínez et.al., 1996; Mahugo Santana et.al., 2009; Nenkova, et.al.2011). Antioxidants can also prevent the loss of food color, flavor and active vitamins content, providing the stabilization of the molecules involved in such characteristics. They can also be used for the production of adhesives and for the synthesis of polymer.

It is well known that, biomass also contains many other natural products: waxes and fatty acids, polyacetylenes, terpenoids (e.g., monoterpenoids, iridoids, sesquiterpenoids, diterpenoids, triterpenoids), steroids, essential oils, phenolics, flavonoids, tannins, anthocyanins, quinones, coumarins, lignans, alkaloids, and glycosidic derivatives (e.g., saponins, glycosides, flavonoid glycosides) (Alonso et.al., 1998; Japón-Luján et.al., 2006; Faustino, 2010; Fang et.al., 2009; Gallo, 2010; Carro, 1997; Kojima, 2004). In this regards, are needed more studies to recover these important compounds from biomass for use in pharmaceutical industry, food industry, and so.

2. Extraction techniques

Actually, there are known many different techniques used for biomass extraction: liquid-solid extraction, liquid-liquid extraction, partitioning, acid-base extractions, ultrasound extraction (UE), microwave assisted extraction (MAE). The capability of a number of extraction techniques have been investigated, such as solvent extraction (J.A. Saunders, D.E. Blume, 1981) and enzyme-assisted extraction (B.B. Li, B. Smith, M.M. Hossain, 2006). However, these extraction methods have drawbacks to some degree.

The choice of extraction procedure depends on the nature of the natural material and the components to be isolated. The main conventional extraction procedures are liquid-liquid extraction and liquid-solid extraction. For liquid-liquid extraction is using two different solvents, one of which is always water, (water-dichloromethane, water-hexane, and so). Some of the disadvantages of this method are: cost, toxicity and flammability (Kaufmann 2002; McCabe, 1956; Perry, 1988; Sarker et. al., 2006).

Solid-phase extraction (SPE) can be used to isolate analytes dissolved or suspended in a liquid mixture are separated from a wide variety of matrices according to their physical and chemical properties. Conventional methods include: soxhlet extraction, maceration, percolation, extraction under reflux and steam distillation, turbo-extraction (high speed mixing) and sonication. Although these techniques are widely used, have several shortcomings: are very often time-consuming and require relatively large quantities of polluting solvents, the influence of temperature which can lead to the degradation of thermo labile metabolites (Kaufmann 2002; McCabe, 1956; Sarker et. al., 2006; Routray, 2012).

Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and pressurised solvent extraction (PSE) are fast and efficient unconventional extraction methods developed for extracting analytes from solid matrixes.

2.1. Supercritical fluid extraction

Supercritical fluid extraction (SFE) is one of the relatively new efficient separation method for the extraction of essential oils from different plant materials. The new products, extracts, can be used as

a good base for the production of pharmaceutical drugs and additives in the perfume, cosmetic, and food industries. Use of SFE under different conditions can allow selecting the extraction of different constituents. The main reason for the interest in SFE was the possibility of carrying out extractions at

temperature near to ambient, thus preventing the substance of interest from incurring in thermal denaturation.

Supercritical fluid extraction has proved effective in the separation of essential oils and its derivatives for use in the food, cosmetics, pharmaceutical and other related industries, producing high-quality essential oils with commercially more satisfactory compositions

(lower monoterpenes) than obtained with conventional hydro-distillation (Ehlers et al., 2001; Diaz-Maroto et al., 2002; Ozer et al., 1996). Also, extraction with supercritical fluids requires higher investment but can be highly selective and more suitable for food products. This plays a mechanistic role in supercritical fluid chromatography (SFC), where it contributes to the separation of the solutes that are injected into the chromatographic system.

Supercritical fluid extraction is an interesting technique for the extraction of flavouring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam distillation processes (Stahl, E. and Gerard, D. 1985). SFE allows a continuous modification of solvent power and selectivity by changing the solvent density (Nykanen, I. et al., 1991). Nevertheless, the simple SFE process, consisting of supercritical CO₂ extraction and a one-stage subcritical separation, in many cases does not allow a selective extraction because of the simultaneous extraction of many unwanted compounds.

2.2. Ultrasound-assisted solvent extraction

Ultrasound assisted extraction is very efficient extraction procedure. Sonication induces cavitation, the process in which bubbles with a negative pressure are formed, grown, oscillated, and may split and implode. By this process different chemical compounds and particles can be removed from the matrix surface by the shock waves generated when the cavitation bubbles collapse. The implosion of the cavities creates microenvironments with high temperatures and pressures. Shock waves and powerful liquid micro jets generated by collapsing cavitation bubbles near or at the surface of the sample accelerate the extraction (R. Kellner et al., 2004). Ultrasonic assisted extraction has many advantages since it can be used for both liquid and solid samples, and for the extraction of either inorganic or organic compounds (S.L. Harper et al., 1983). If extracted from solid samples, different problems can occur: there is a possibility of the decomposition of the analyte which could be trapped inside of the collapsing cavitation bubbles. The ultrasound extraction system can be also applied as a dynamic system in which the analytes are removed as soon as they are transferred from the solid matrix to the solvent. In this process, furthermore, the sample is continuously exposed to the solvent (I. Rezić' et al., 2008).

This is a modified maceration method where the extraction is facilitated by the use of ultrasound (high-frequency pulses, 20 kHz). Ultrasound is used to induce a mechanical stress on the cells of biomass solid samples through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent and improves extraction yields. The efficiency of the extraction depends on the instrument frequency, and length and temperature of sonication. Ultrasonification is rarely applied to large-scale extraction; it is mostly used for the initial extraction of a small amount of material. It is commonly applied to facilitate the extraction of intracellular metabolites from plant cell cultures (Kaufmann, 2002; Sarker, 2006).

2.3. Pressurized Solvent Extraction (PSE)

Pressurized solvent extraction or “accelerated solvent extraction,” employs temperatures that are higher than those used in other methods of extraction, and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples. The solid biomass sample is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material. High temperatures and pressures increase the penetration of solvent into the material and improve metabolite solubilization, enhancing extraction speed and yield. Moreover, with low solvent requirements, pressurized solvent extraction offers a more economical and environment-friendly alternative to conventional approaches

As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity. An additional advantage is that the technique can be programmable, which will offer increased reproducibility. However, variable factors, e.g., the optimal extraction temperature, extraction time, and most suitable solvent, have to be determined for each sample (Kaufmann, 2002; Tsubaki, 2010; Sarker, 2006).

Microwave-assisted extraction (MAE) or simply microwave extraction is a relatively new extraction technique that combines microwave and traditional solvent extraction. The microwave energy has been investigated and widely applied in analytical chemistry to accelerate sample digestion, to extract analytes from matrices and in chemical reactions. Application of microwaves for heating the solvents and plant tissues in extraction process, which increases the kinetic of extraction, is called microwave-assisted extraction. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles, without changing the molecular structures if the temperature is not too high. Nonpolar solvents, such as hexane and toluene, are not affected by microwave energy and, therefore, it is necessary to add polar additives. Microwave-assisted extraction (MAE) is an efficient extraction technique for solid samples which is applicable to thermally stable compounds accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from materials. The microwave-assisted extraction technique offers some advantages over conventional extraction methods.

Compared to conventional solvent extraction methods, the microwave-assisted extraction (MAE) technique offers advantages such as improved stability of products and marker compounds, increased purity of crude extracts, the possibility to use less toxic solvents, reduced processing costs, reduced energy and solvent consumption, increased recovery and purity of marker compounds, and very rapid extraction rates.

The use of MAE in natural products extraction started in the late 1980s, and through the technological developments, it has now become one of the popular and cost-effective

extraction methods available today, and several advanced MAE instrumentations and methodologies have become available, e.g., pressurized microwave-assisted extraction (PMAE) and solvent-free microwave-assisted extraction (SFMAE).

Comparison between conventional and MAE extraction method

This technique has been used successfully for separation of phenolic compounds from types of biomass, polyphenols derivatives, pyrimidine glycosides, alkaloids, terpenes, and so.

In most cases, the results obtained suggested that the microwave assisted method was more convenient even compared to the ultrasound extraction method.

Pyrimidine glycosides

The studies regarding the microwave extraction of vicine and convicine (toxic pyrimidine glycosides) from *Vicia faba* using a methanol: water mixture (1:1 v/v) involves two successive microwave irradiations (30 s each) with a cooling step in between. No degradation could be observed under these conditions, but further irradiation was found to decrease the yield of vicine and convicine. The yield obtained was 20% higher than with the conventional Soxhlet extraction method.

Alkaloids Sparteine, a lupine alkaloid, was extracted from *Lupinus mutabilis*, with methanol: acetic acid (99:1, v/v) in a common microwave oven and the microwave irradiation program used one to five cycles of 30 s with a cooling step in between and conduct to 20% more sparteine than was obtained with a shaken-flask extraction using the same solvent mixture for 20 min.

Terpenes Five terpenic compounds: linalool, terpineol, citronellol, nerol and geraniol, associated with grape (*Vitis vinifera*) aroma was extracted from must samples by MAE (Carro et al., 1997). Was investigated the influence of the parameters: extracting solvent volume, extraction temperature, and amount of sample and extraction time. Several conditions were fixed, such as the extraction time (10 min) and the applied power (475 W). The solvent volume appeared to be the only statistically significant factor, but was limited to 15 mL by the cell size. The highest extraction yield was obtained with both the solvent volume and the temperature at their maximum tested values. In contrast, the sample amount had to be minimized in order to obtain the best recoveries. The final optimized extraction conditions were as follows: 5 mL sample amounts extracted with 10 mL of dichloromethane at a temperature of 90°C for 10 min with the microwave power set at 50% (475 W).

Steroids Recently, was demonstrated that only 30–40 s were sufficient to extract ergosterol quantitatively by MAE using 2 mL methanol and 0.5 mL 2 M sodium hydroxide. Microwave irradiation was applied at 375W for 35 s and the samples were cooled for 15 min before neutralization with 1 M hydrochloric acid followed by pentane extraction. The yield was similar to or even higher than that obtained with the traditional methanolic extraction followed by alkaline saponification and pentane extraction.

Alkaloids The extraction of two alkaloids cocaine and benzoylecgonine by focused MAE was optimized by taking into account several parameters such as the nature of the extracting solvent, particle size distribution, sample moisture, applied microwave power and radiation time. MAE was found to generate similar extracts to those obtained by conventional SLE but in a more efficient manner. Indeed, 30s were sufficient to extract cocaine quantitatively from leaves, using methanol as solvent and a microwave power of 125 W. (Kaufmann, 2002).

Phenolic compounds

In recent years, synthetic antioxidants were reported to have the adverse effects such as toxicity and carcinogenicity and this situation has forced scientists to search for new natural antioxidants from herbs or the other materials. Phenolic compounds, the most important bioactive compounds from plant sources, are among the most potent and therapeutically useful bioactive substances, providing health benefits associated with reduced risk of chronic and degenerative disease (Luthria, 2006; Tsubaki et al., 2010; Proestos, 2008).

Extraction is one of the most imperative steps in the evaluation of phenolic compounds from plant. Often is done a saponification prior to the extraction step because is necessary to cleave the ester linkage to the cell walls (Robbins, 2003).

The capability of a number of extraction techniques have been investigated, such as solvent extraction and enzyme-assisted extraction. However, these extraction methods have drawbacks to some degree. For example, solvent extraction is time consuming and enzyme in enzyme assisted extraction is easy to denature. In the case of Soxhlet extraction, the extraction time vary from 1 minute to 6 h. Ultrasonic is one of the most industrially used methods to enhance mass transfer phenomena (Japón-Luján et.al. 2006; Luthria, 2006; Pérez-Serradilla, 2007). Meanwhile, microwave assisted extraction heats the extracts quickly and significantly accelerates the extraction process (Martínez, 1996; Kojima, 2004; Patsias, 2009). Simultaneous ultrasonic/microwave assisted extraction (UMAE) coupled the advantage of microwave and ultrasonic, presenting many advantages (Kojima, 2004).

Extraction of phenolic compounds from solid samples is usually carried out by stirring (Luthria, 2006; Nepote, 2005), although the use of auxiliary energies has demonstrated to accelerate the process (Japón-Luján, et.al.2006; Pérez-Serradilla, 2007). Microwave-assisted extraction (MAE) is the process by which microwave energy is used to heat polar solvents in contact with solid samples and to partition compounds of interest between the sample and the solvent, reducing both extraction time and solvent consumption.

The conventional liquid–solid extraction techniques, such as heat reflux extraction (HRE), ultrasonic extraction (UE) and maceration extraction (ME), are discommodious, laborious, time-consuming and require large volumes of toxic organic solvents. So increasing attention is paid to the development of more efficient extraction methods for the rapid extraction of active compounds from materials.

The current analytical methods used to extract phenolic compounds from liquid samples are based on *liquid-liquid extraction* (LLE). Although this technique offers efficient and precise results, it is relatively time-consuming, possibly harmful due to the use of large volume of organic solvents (frequently toxic) and highly expensive. For these reasons, there is an increasing tendency to replace LLE by solid-phase extraction (SPE) for liquid samples. SPE was developed in the 1980s, and has emerged as a powerful tool for chemical isolation and purification. This methodology is an alternative extraction to LLE due to it reduces organic solvents consumption, the length of analysis and it can be automated (Martínez, et. al., 1996; Kojima, 2004; Patsias, et.al., 2009).

Although most attention has been focused on the determination of phenolic compounds in aqueous samples, more substituted phenols, such as pentachlorophenol, show limited transport in water and they are more likely absorbed in sediments and soils. This fact contributes to the persistence of these compounds in the environment and it results in high concentrations of them that could affect aquatic and earth organisms. For extraction, Soxhlet extraction is one of the most popular techniques for isolating phenolic compounds from solid samples, due to its simplicity, inexpensive extraction apparatus. Despite the good results obtained with this methodology, Soxhlet extraction makes the analysis procedure excessive time consuming. Moreover, it requires large amount of hazardous organic solvents.

Ultrasonic extraction is another conventional technique to extract analytes from solid samples. Although sonication is faster than Soxhlet extraction, it also requires large volumes of toxic and expensive organic solvents.

The studies show that the compounds are extracted more effectively when the energy provided by microwave is employed (Perez-Serradilla, 2011).

3. Experimental studies

The efficiencies of different solvents (water, acid and alcohol) in the extraction of caffeine and phenols from leaves of white, black, green and red tea in different solvents: ethanol, isopropanol, methanol and water. Extraction was performed comparative by ultrasonic and by MAE. Determination of the total amount of phenolic compounds was studied comparative using different extraction times 5, 15 and respectively 30 minutes. The microwave irradiation shortens time necessary to extract phenols and caffeine from tea samples (between 30 and 50 seconds). The results of the comparison investigation are presented in the figure 1.

4. Conclusion

Chromatographic determination of phenolic compounds isolated from the tea samples by ultrasonic and MAE extraction is comparable. The difference between the two methods of extraction consists in extraction time and amount of solvents used. Also, the yield for MAE was about 20% is 20% higher than that of the ultrasonic extraction.

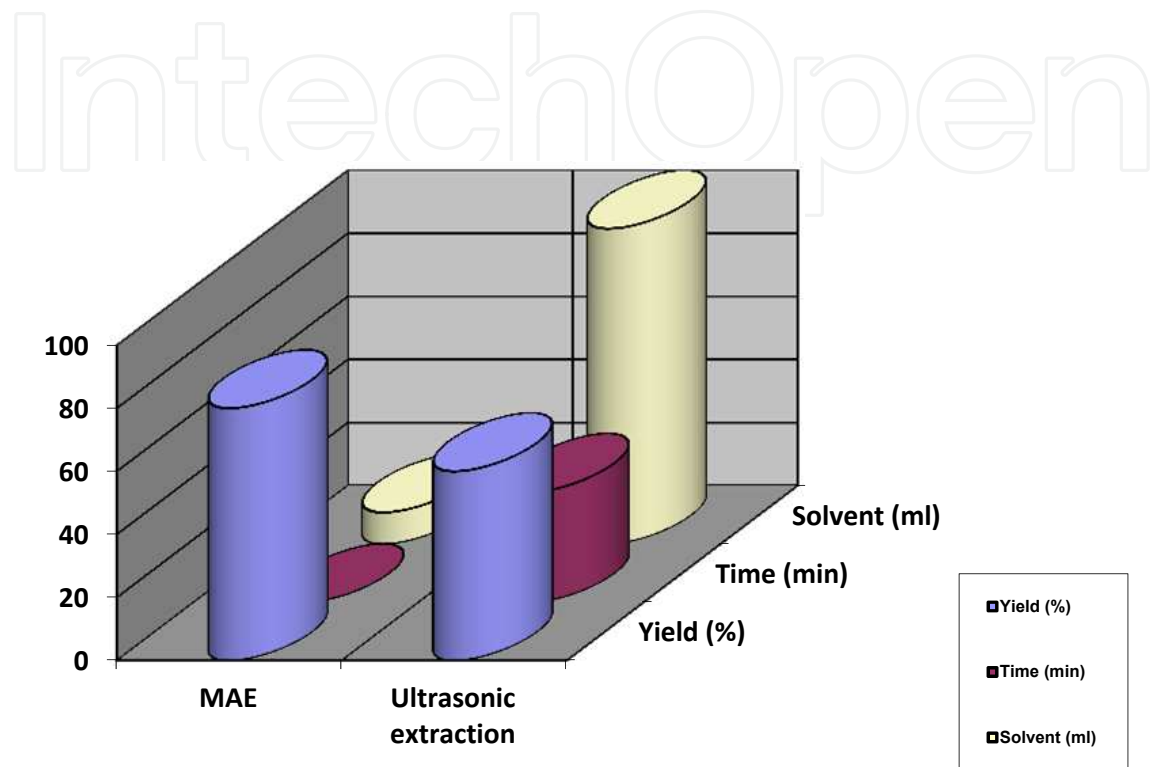


Figure 1. Comparison between the two extraction methods

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