THE EXTRACTION OF VALUABLE COMPOUNDS FROM STRAW DEGRADED BY *PLEUROTUS OSTREATUS*

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ABSTRACT

This work aims to the recovery of lignocellulosic waste in an environmentally friendly process, as an alternative to the energy- intensive technologies: steam explosion, subcritical and/or supercritical water treatment, gasification through pyrolisis, etc.

A study was made to optimize the extraction conditions of potentially valuable compounds in straw degraded by the fungus *Pleurotus ostreatus*. The effects of solvent nature, temperature and extraction time were quantified by material balances with a special view to the extracts obtained. Confirmation of the effectiveness of the operations was done by spectrophotometric, HPLC and LC-MS analyses.

Following this study, a technology localized to the farm was conceived, requiring few craftsmanship and no special utilities, to obtain a semi-product for further processing. A centralized technology could be also taken into account to process the straw by direct extraction with hot solvents, in order to obtain products yields three times higher than in the case of the aqueous extraction followed by solvent extraction at 20^{o}C.

Keywords: agricultural waste, environmentally friendly process, *Pleurotus ostreatus*, lignocellulose, extraction

1. Introduction

Large amounts of agricultural waste arises the disposal problem every year. As an alternative, the biorefineries can take over a part of this waste, producing fuel, side products, heat and power, the major stint of this approach being the cost of the transportation from the farms to the biorefinery [1]. There is also the possibility to use agricultural waste in local facilities for producing biogas or bioethanol.

Studies conducted in the last 30 years have shown that industrial applications may result from lignocellulosic degradation research (Crawford and Crawford, 1980). Fungal submerged fermentation (Bjerre et al., 2000; Milstein et al., 1981; Mishra and Pandey Lata, 2007; Ruffell et al., 2010) or solid state fermentation (Dinis et al., 2009; Hernández-Coronado et al., 1997; Valmaseda et al., 1991a, 1991b) have been extensively studied. The fungal attack on lignocellulosic feedstock was taken into account as a pretreatment of biomass because of its potential to lower the severity of further thermocatalytical biomass processing (Akin et al.,1993; Karunanandaa et al.,1992; Keller et al.,2003). This had in view the biofuel production using fungal pretreatment. Some edible mushrooms can also be used for the pretreatment of lignocellulosic feed for animals, improving the rumen digestability, as a consequence of the breakdown of the polymeric lignin structure by the enzymes (Agosin and Odier, 1985; Basu et al., 2002; Hadar et al., 1993; Kerem and Hadar,1992). But straw degraded by fungi is also an energy feedstock and a source of valuable compounds for novel chemicals *e.g.* C_5 and C_6 sugars, phenolics (Burton and Eastwood, 2009; Hassan et al., 1991). Considerable potential exists for producing such low molecular weight chemicals. This approach gains ground when trying to reduce the dependency on petroleum as a resource for chemical synthesis.

This work aims to the recovery of lignocellulosic waste in an environmentally friendly process, as an alternative to the energy-intensive technologies. This process can potentially be performed locally, at farm scale. In the authors' view, it is a process consisting of a two stage extraction: an aqueous extraction followed by a solvent extraction, in mild conditions. The aqueous extract can be used as an energy feedstock, for the production of biogas; the separation of some phenolics from the aqueous extract could be also taken into account. The interesting chemicals in the solvent extract can be concentrated and sold to be used in further syntheses.

2. Materials and methods

A laboratory study aimed to find the optimum conditions for the extraction of valuable compounds from straw. The study consisted on aqueous and solvent extractions applied to straw degraded by *Pleurotus ostreatus* mycelium grown for 6 month during the summer season.

Aqueous extraction was performed at 20^oC, 40^oC, 60^oC and 80^oC.

Around 70 g straw weighed with 0.1 g precision, was put into a 500 mL flask with water (400 mL), and kept under stirring long enough to reach the extraction equilibrium. The extraction time was determined by measuring the sugars and phenols levels in time and observing when they reached a plateau. Also, the extraction time was checked with the evolution of the Easy Oxidable Organic Carbon. EOOC gives an indication of the organic matter extracted by a fast titrimetric method for soils analysis which was adapted for aqueous extracts.

The extract was filtered through muslin and cellulose-free filter. Phenolics and sugars concentration in the extract was measured by UV-VIS absorption spectrometry. The material

balance allowed us to express the effectiveness of the operations in terms of the extracted matter per kg dry straw and losses.

The squeezed straw was transferred into 250 mL flasks and solvent (200 mL) was added. The solvents used in this study were ethanol, dichloromethane, toluene and mixtures of them.

The solvent extraction took place at 20°C, for 19 hours, under shaking. Then, the solution was filtered under vacuum in a Buchner funnel, through cellulose-free filter pore size 7.0 µm. The cake was transferred on aluminium foil, weighed for mass balance purposes, dried for 1-2 days, in the fume cupboard, then in the oven at 100°C, at constant weight. The solvent was removed from the filtrate under vacuum. The dry extract was transferred to vials, rinsing the flask with 5-6 mL of CH_2Cl_2/MeOH (LC-MS grade). The vials were kept open in a fume cupboard, allowing solvent to evaporate. The dry matter was analysed qualitatively by LC-MS for the identification of interesting compounds. Material balances on solvent extract operations served to measure the extracted matter per kg dry straw and losses during this operation.

For water and organic solvent extractions over 60°C, the DIONEX apparatus for accelerated extraction was also used. For this experiment the machine was programmed to work at 100 bar and 60 °C, 80°C, 100°C and 150°C respectively.

With the DIONEX apparatus, the effect of the temperature for both water and organic solvent extractions was checked. Also, direct extractions with organic solvents (without a previous aqueous extraction) at 150°C were performed.

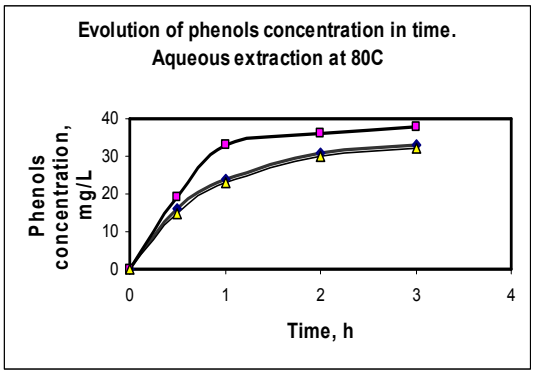
Following this study, a technology localized to the farm was conceived, requiring few craftsmanship and no special utilities, to obtain a semi-product for further processing.

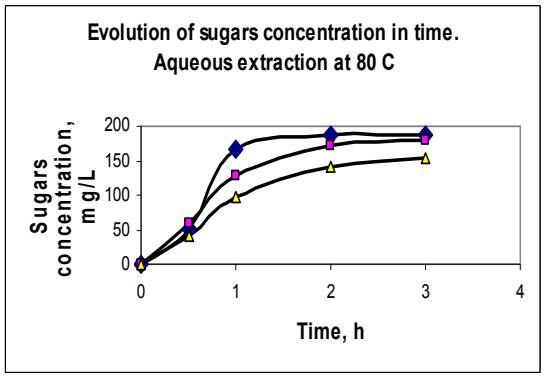
3. Results and discussion

3.1. Results of the laboratory study

3.1.1. The optimum time for the aqueous extraction operation

In order to recover as much matter as possible, the extraction time should be long enough to reach the extraction equilibrium but not exceeding this. The optimum time was found when the sugars, phenols and EOOC reached a plateau, as exemplified in Figure 1, for the extraction at 80°C.





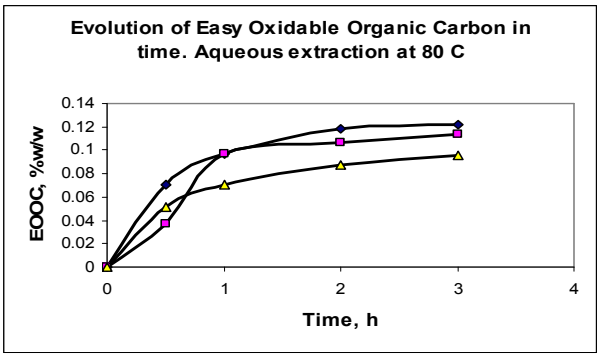


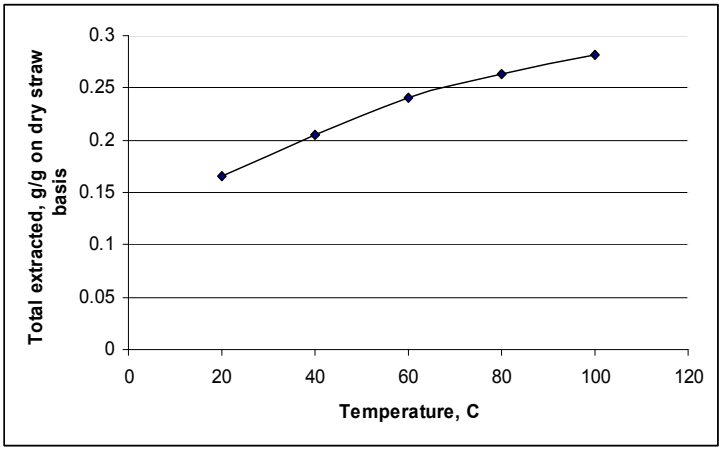
Fig.1. Determination of the optimum extraction time at 80°C

As seen in Figure 1, the optimum time for the aqueous extraction at 80°C is 2 hours. Similarly, the determined optimum time at 20°C and 40°C was 15 h and at 60°C was 3 hours. That time can be overrun but this will cause higher energy consumption.

3.1.2. The effect of the aqueous extraction temperature

A large amount of material was recovered by aqueous extraction (0.16-0.28 g/g dry straw), containing organic and inorganic compounds. The extracted mass obtained from the aqueous extract increases with the extraction temperature (Figure 2). Also, the losses calculated by mass balance increased constantly with temperature, from 1.4% at 20°C to 11.8% at 80°C; these losses are due mainly to water evaporation but this can be minimized by operating a closed system.

The trend for phenols and sugars quantities extracted per g dry straw is increasing with temperature, in general (see Figure 2).



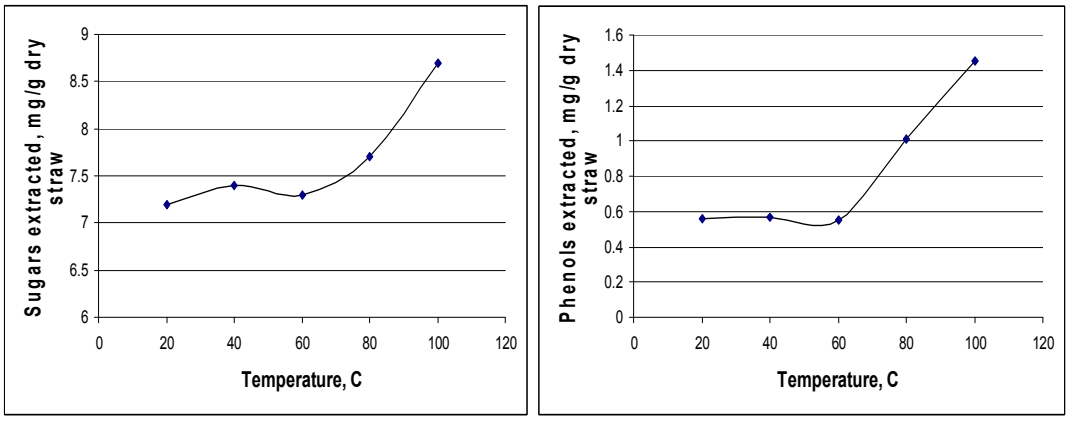


Fig.2. Influence of temperature on total amount respectively sugars/ phenols extracted in water

From Figure 2, it can be observed that in the range 20-60^{o}C, comparable quantities of sugars and phenolics are extracted, since over 60^{o}C, the increasing is obvious.

3.1.3. The effect of the solvents used

The solvent extractions were performed either manually, at 20^{o}C, after the water extraction at 20^{o}C, 40^{o}C, 60^{o}C, 80^{o}C, or by the Dionex apparatus at higher temperature (80^{o}C, 150^{o}C). The study allowed us to conclude about the optimum solvent combination and the effect of the solvent temperature.

Pure solvents (ethanol, dichloromethane and toluene) and mixtures of ethanol with dichloromethane and toluene respectively in 25%, 50% and 75% proportion were used. Among the three pure solvents, the ethanol extracted more matter but mixture of solvents were even better. In Figure 3, the results of the extraction at 20^{o}C with different solvents and mixtures are shown, following the aqueous extraction at 20^{o}C. The most is extracted by the mixtures 50%-50% vol/vol ethanol/ dichloromethane or toluene. It can be also observed that the mixtures ethanol/dichloromethane extract more than ethanol/toluene. Similar results were obtained when previous aqueous extraction was performed at 40^{o}C, 60^{o}C and 80^{o}C.

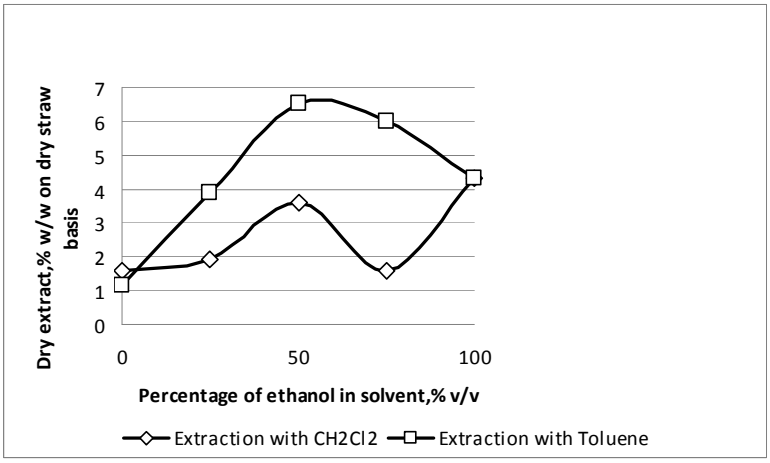


Fig.3.The influence of the solvent on the total extracted matter at 20^{o}C

3.1.4. The influence of the solvent temperature

The increasing of the temperature leads to higher amount extracted, whatever the solvent is. This is illustrated in Figure 4 for different solvents extraction at 80°C and 20°C respectively, following the aqueous extraction at 80°C.

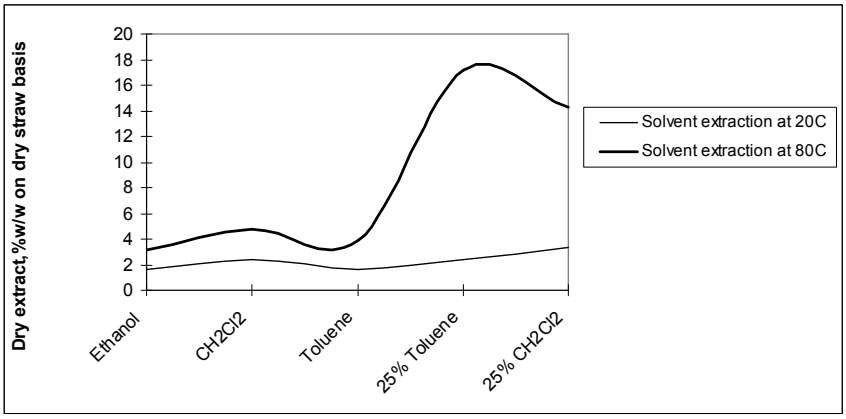


Fig.4.The influence of the temperature on total extracted matter in solvent

From figure 4, it also can be seen that at 80°C, the mixture of ethanol with dichloromethane or toluene are neat superior to the pure solvents since the dry extract yield is 3-4 time greater.

3.1.5. The direct extraction with organic solvents

Direct solvent extractions were performed manually at 20°C, as described in Section 2, paragraph 5 or with the Dionex apparatus, at 150°C. The results are shown in Table 1, comparatively with the solvent extraction preceded by aqueous extraction. Higher yields of extract are obtained by direct solvent extraction even at 20°C comparing with organic solvent extraction anteceded by aqueous extraction. It can be explained by the water remaining in straw after the aqueous extraction and squeezing. The diffusion of the solvent in the fibres becomes more difficult since water adheres to them.

Table 1. Direct solvent extraction compared with that anteceded by aqueous extraction

Solvent	Extracted in solvent at 20°C following aqueous extraction at 20°C, % w/w	Extracted directly by solvent at 20°C, %w/w	Extracted directly by solvent at 150°C, %w/w
EtOH	4.3	6.1	20.0
CH_2Cl_2	1.6	4.5	11
Toluene	1.2	3.4	4.8
25% CH_2Cl_2+75%EtOH vol/vol	1.6	5.6	13.6
25% Toluene+75%EtOH vol/vol	6.0	6.6	14.5

From Table 1, one can conclude that extraction with hot solvents is several times more efficient than at environmental temperature. This would be an operation performing at high pressure so it could be taken into account only for a centralised industrial process design.

3.1.6. Valuable compounds identified in the solvent extract

The solvent extracts were analyzed by LC-MC. Chromatograms are complex, however they have common features: 2-4 peaks at the retention times between 3 and 4 min,

than a big fat peak between 14 and 17, composed in fact by 3-4 peaks pasted together. There is an important peak around 21 min and many smaller peaks between 25 and 44 minutes. Some of the differences between chromatograms can be assigned to the solvent used at the extraction. For example, there were observed distinctive peaks at R_T=11.1-11.5 with bigger abundance for samples proceeding from the extraction with toluene. The dichlorometane extracts give higher peaks at R_T=15.2, 15.7, 16.2. The ethanol extracts have a chromatogram with many peaks at longer retention times.

Some compounds were identified with standards (Figure 5), by comparing the retention times and the MS spectra of the samples and the standards.

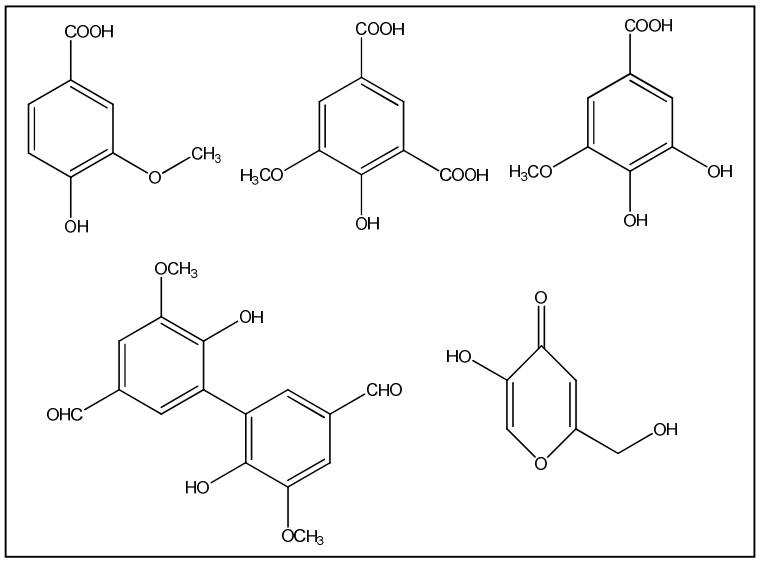


Fig.5. Compounds identified in some extracts in organic solvents

The phenolic acids are valuable compounds because they are the raw for further synthesis of resins for sustainable composite materials.

3.2. Working out a process technology

The raw for this process is the wheat straw partially decomposed by Pleurotus ostreatus after the mushroom growing and the harvesting (Bisaria at al.,1987). Advanced degradation can enable significant quantities of the molecules of interest to be recovered. This process can potentially be performed locally, at farm scale. A simple process for recovery would consist on aqueous extraction followed by organic solvent extraction and the separation of valuable compounds from the extract (see Figure 6).

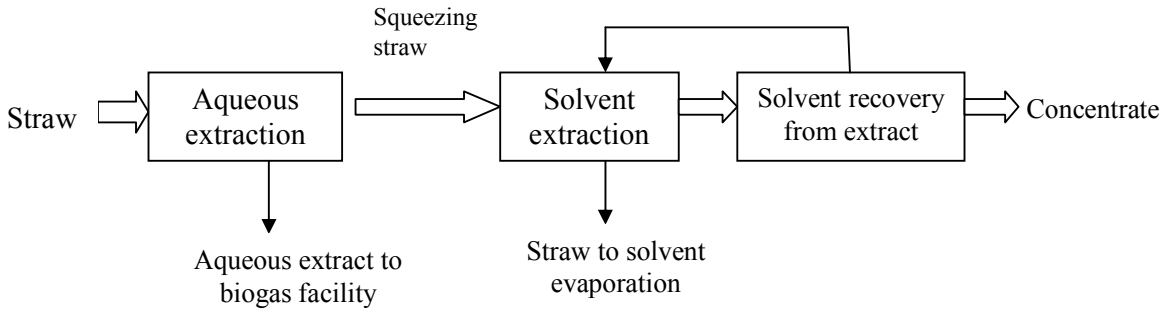


Figure 6. Local process technology for the recovery of valuable products from straw degraded by *P.ostreatus*

The aqueous extraction phase can be seen as a recovery operation of some organic matter for the production of biogas. Recovery of sugars and phenols from the solution wouldn't be an option since their concentration level is low (max. 400 mg/L sugars and max. 50 mg/L phenolics) and the production of these, calculated on dry-weight basis, is max.8 mg sugars/g straw and max.1 mg phenolics/g straw. But the main reason for performing aqueous extraction is to remove water soluble compounds and make way for the solvent to extract lignin-degraded compounds.

Aqueous extraction at higher temperature is more efficient from the viewpoint of the extracted matter, but for an environmentally friendly process, less energy, including heating would be consumed. It is to see if power consumption for agitation during 15 hours at 20^{o}C would exceed that for heating and agitation during 2 hours at 80^{o}C; it depends on local costs of these operations.

An aqueous extraction at 20^{o}C is feasible, since the quality of the extract is of secondary interest and this stage could be seen only as a previous step for the organic solvent extraction, where valuable compounds are expected to be recovered. A mixture containing up to 25% dichloromethane and ethanol is more efficient at solvent extraction. For safety and toxicology reasons, the preferred solvent is the ethanol.

After the aqueous extraction, the water would be removed from straw, for a better solvent extraction, as discussed in Section 3.1.5. A very efficient way to do this is by freezing the straw followed by water sublimation, but this is an energy-costly operation, so the squeezing would be a more environmentally friendly option, accepting that lower extraction yields would be obtained as a consequence.

Although higher temperature solvent extraction gives higher mass and more phenolic extracts, the economics and safety considerations means that an ambient temperature extraction is more realistic for a localised process tehnology.

The solvent extract is then filtered and the straw would retain up to 10% of the solvent used in the operation. The solvent from straw would be evaporated in the air in a special tank, provided with vapor condenser on airing, for the solvent recovery.

For a simple operating mode, the following operations could be performed in the same tank: water extraction, aqueous extract filtration, squeezing of straw, solvent extraction, solvent extract filtration and evaporation of the solvent from straw.

In the present study, the solvent recovery from extract was performed by vacuum evaporation. This is a slow process, difficult to operate and energy-costly. A simpler operation would be the recovery of the solvent with separation membranes. A carefully chosen membrane and the right separation parameters would make this operation easy to perform, with no special skills.

The concentrate in Figure 6 contains the interesting compounds for chemical syntheses, a semi-product of high value and small volume.

4. Conclusions

A study was carried out to optimize the extraction conditions of potentially valuable compounds in straw degraded by the fungus *Pleurotus ostreatus*. The effects of solvent, temperature and extraction time were quantified by mass balance with a view to the extracts obtained. Confirmation of the effectiveness of the operations was conducted by LC-MS

The conclusions of the study were the following:

- It is possible to have a process using aqueous extraction at 20^{o}C followed by an organic solvent extraction at 20^{o}C. In this case, the aqueous extracts only contain relatively low levels of total organic compounds (TOC), These levels are such that they have limited use for obtaining biofuels (*e.g.*biogas, bioethanol);
- For the solvent extractions at 20^{o}C following the 20^{o}C aqueous extraction, the highest level of organic extracts were achieved using mixtures of dichloromethane with ethanol;
- To establish a working process at atmospheric pressure and considering environmental, health and safety issues, dichloromethane should be limited to 25% volume of total solvent used;
- High extractible yields are obtained if the aqueous extraction is performed at higher temperature. At 60^{o}C and at 80^{o}C, higher levels of TOC are obtained from aqueous extracts, at levels that could form part of the raw material for a local biogas installation;
- The final decision on the aqueous extraction temperature would be taken after the analysis of the costs with mixing power and heating, taking into account that the optimum time for the extraction at 20^{o}C is 15 hours, since at 80^{o}C, it is only 2 hours;
- For the solvent extractions at 20^{o}C following the 60^{o}C and 80^{o}C aqueous extraction, the gratest mass was extracted with neat ethanol. However, LCMS showed more phenolics were extracted with 25%DCM+ 75% EtOH;
- Although higher temperature solvent extraction gives higher mass and more phenolic extracts, the economics and safety considerations means that an ambient temperature extraction is more realistic for a localised process tehnology;
- A centralised technology could be also taken into account to process the straw by direct extraction with hot organic solvents, in order to obtain products yields three times higher than in the case of the aqueous extraction followed by solvent extraction at ambient temperature.

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