

New analysis method of furfural obtained from wood applying an autohydrolysis pretreatment

Received for publication, August 20, 2012

Accepted, October 20, 2012

LACRIMIOARA SENILA*, MIRELA MICLEAN, MARIN SENILA,
MARIUS ROMAN, CECILIA ROMAN

INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Str.,
400293, Cluj-Napoca, Romania, Tel: +40-264-420590, Fax: +40-264-420667, E-mail*:

Corresponding authors: lacri.senila@icia.ro

Abstract

Two gas chromatography extraction methods based on liquid-liquid (LLE) and headspace solid phase microextraction (HS-SPME) were evaluated for analysis of furfural from hemicellulose hydrolysates. Woody biomass was used as renewable resource for furfural production. In this study, autohydrolysis pretreatment was investigated for conversion of xylose and arabinose into furfural at selected ranges of temperatures (180-200°C) and time (5-15 min). The autohydrolysis pretreatment performed at temperature of 200°C and 15 min gives the high furfural content. The maximum pentose and hexose concentration was obtained at 190°C for 10 min reaction time. The results show that the HS-SPME extraction provides better results comparatively with LLE extraction method: no chemicals use; good recovery (95%); relative standard deviation (RSD) value is 7%, showing a good precision of the method; lower detection limits (0.03 µg ml⁻¹); all these showing that the method can be applied for determination of furfural from wood hydrolysates.

Keywords: wood, furfural, solid phase microextraction, GC-MS

Introduction

Furfural is a chemical product used in the oil refineries, plastics, food, pharmaceutical and agricultural industries or as intermediary chemical for production of tetrahydrofuran, 2-methyltetrahydrofuran and furfuryl alcohol [1].

Furfural is produced by removing water from or dehydrating five-carbon sugars or, by acid hydrolysis of lignocellulosic biomass, such as: corncobs, wheat straw, sugarcane bagasse, cotton stalks and wood (wood chips) [2, 3].

Lignocellulosic biomass gains a great attention due to the high content of carbohydrates. Lignocellulosic biomass contains approximately 42-54% cellulose, 23-36% hemicellulose and 22-28% lignin. Cellulose, a polymer of glucose and hemicellulose, is composed from pentose and hexose [4]. Hemicellulose from wood is the most abundant polymer from lignocellulosic biomass containing pentose (xylose and arabinose), and can be used as raw material to obtain different chemical products: acids (succinic, itaconic, levulinic, glucaric, aspartic, 2,5-furandicarboxylic, glutaric, 3-hydroxypropionic, 3-hydroxybutyric acid beta-lactona), sorbitol, xylitol and glycerol and furfural [5, 6].

The pentose from hemicellulose can be converted into furfural during the pretreatment method - proving that woody biomass represents a renewable source for furfural production.

The first step in obtaining the above mentioned products is the separation of hemicellulose from wood and then, its hydrolysis to monosaccharide. Separation of hemicellulose could be made using chemical, biochemical, physical or physico-chemical methods. The usual pretreatment methods used for hemicellulose separation are: steam explosion, organic solvents, acid or alkali treatments and autohydrolysis. The majority of methods uses chemicals, requires long time processed and additional steps for purifications [7].

The methods used for extraction of furfural by GC-MS are liquid-liquid extraction, solid phase extraction, and simultaneous distillation liquid extraction [8]. Headspace solid phase microextraction (HS-SPME) is rapid, has a high selectivity and sensibility; it is simple, low cost and short time of extraction, eliminates the use of organic solvents, and extraction and pre-concentration are made in a single run [9]. The SPME contains a fiber coated with polymeric stationary phase introduced in headspace of a liquid sample. One of its advantages is its simplicity.

Pentose monomers under acidic condition can degrade to furfural [10]. Autohydrolysis pretreatment of wood was used for extraction of pentose from wood, with a pressure reactor at high temperatures [11]. Water was used as solvent for separation of pentose. Due to high pressure and temperature, the hemicellulose fraction is degraded into sugars composed of mainly pentose and some hexoses. Autohydrolysis presents many advantages: it uses only high pressure and temperature, no uses chemicals, has high yield recovery of hemicellulose and the hemicellulose is recovered as oligosaccharide, monosaccharide and furfural compounds [12]. Formation of furfural during autohydrolysis is function of reaction conditions (temperature, pressure, time of reaction). Due to its advantages, the autohydrolysis method of wood to produce furfural could be a good alternative to classical methods [6, 13, 14].

The hemicellulose fraction resulted after extraction contains sugars and non-volatile compounds (the byproducts resulted after lignin degradation). The characterization/analysis of furfural represents a mandatory step of the process of furfural obtaining. The usage of the appropriate method of analysis must take into account that the furfural is an organic compound with high volatility and lipophilicity [9]. The existing methods for furfural analysis are either classical methods, as colorimetry and spectrometry, or advanced techniques, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) [15, 16, 17], the methods mentioned presenting both advantages and disadvantages.

The purpose of this paper is to compare two analytical methods used for furfural extraction from hemicellulose fraction resulted after autohydrolysis of wood: liquid-liquid, and headspace SPME extraction. The furfural compound was determined by gas chromatography coupled with mass spectrometry.

Materials and methods

Chemicals and samples

All chemicals were analytical reagent grade. Furfural solvent (98%) was purchased from Alfa Aesar (Germany). Dichloromethane, sodium chloride (NaCl, 99%), ammonium sulfate were purchased from Merck (Darmstadt, Germany). Sodium chloride was used to decrease the solubility of furfural and increase adsorption of analytes by SPME technique. A concentration level of 30 g L⁻¹ NaCl was selected, according to Nabarlantz studies [13]. Stock standard solutions of furfural (1160 mg l⁻¹) were prepared in water and stored at 4°C by dissolved 25 µl furfural solvent in 25 ml water. Working solutions were prepared by appropriate dilution of the stock standard solutions with ultrapure water. Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Only external calibration standards were used. The pH was determined using a pH meter (pH meter Jenway 3340).

Autohydrolysis of wood

For the formation of furfural, approximately 25 g wood was put in a Parr reactor (Parr Instruments, Illinois, USA, 1 L) and 175 ml water was added. Other works show that influence of content solid/liquid between 7 and 10 g water/g raw material is negligible [18]. After the reacting mixture at the desire temperatures and times, the mixture of reaction was

cooling and separated by filtration. The operations were carried out at three different temperatures: 180, 190 and 200°C for 5, 10 and 15 min reaction time, tested for each temperature. Pressure of 60 bar was used in all experiments. The liquid fraction was used for furfural and sugars analysis. The experimental procedure used to convert wood to furfural is shown schematically in Figure 1.

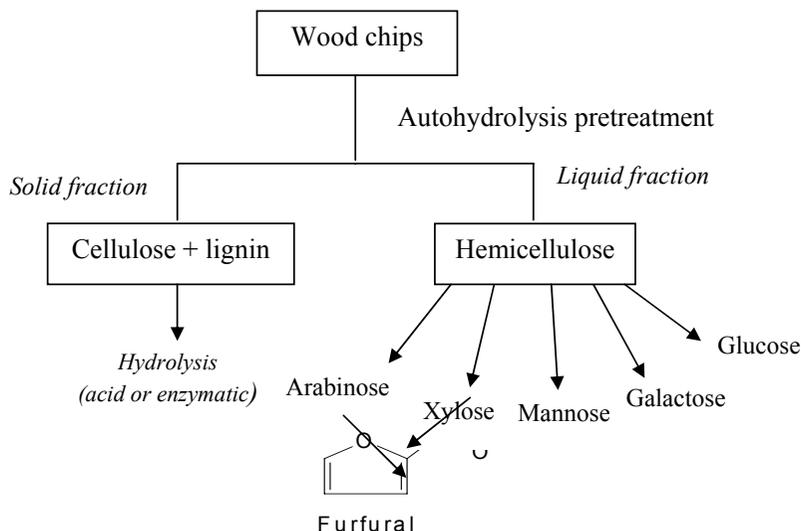


Figure 1. Overview of experimental scheme

Liquid-liquid extraction of furfural

Ten milliliters of standard or samples and 3 g ammonium sulfate were extracted with 10 ml dichloromethane. The pH was adjusted to 4 by adding amount of 8% sodium hydroxide solution. The mixture was centrifuged (10 min at 4500 rpm). The organic phase was separated and dried with sodium sulfate. The extract was concentrate to 0.5 mL and then was injected in GC-MS.

Headspace solid phase microextraction (HS-SPME) of furfural

Ten milliliter of standard or sample was placed in a 20 ml headspace vial and sealed with a PTFE/Silicone septum and then, it was placed in a water bath. 30 g L⁻¹ NaCl and a magnetic stirrer bar for samples were put in a vial sealed. The extractions were performed with the exposure of the DVB/CAR/PDMS (Supelco) fiber to the headspace of the sample for 120 min at 25°C, under constant stirrer (120 rpm). The pH of the sample was adjusted to 4 with NaOH with a pH-meter. After extraction, the fiber was removed from the sample and introduced in the GC injector for 5 min at 250°C for thermal desorption.

GC-MS conditions

A gas chromatograph 6890N (Agilent Technologies) coupled with a mass spectrometer 5973N MSD (Agilent Technologies) and a capillary column HP-5 MS (30 m×0.25 mm×0.25 μm) were used to analyze the furfural extracted by LLE and HS-SPME extraction methods. The carrier gas was helium with a constant flow rate of 1 mL min⁻¹. The injector temperature was 250°C, and the splitless injection mode was used. Samples were analyzed in EI full-scan data acquisition over the range m/z of 50-300. The temperature program was the following: initial oven temperature set at 50°C, held for 1 min, followed by an increasing of temperature

with a rate of $2.5^{\circ}\text{C min}^{-1}$ to 100°C , temperature increase of $2^{\circ}\text{C min}^{-1}$ to 180°C , followed by increasing of temperature with a rate of $15^{\circ}\text{C min}^{-1}$ to 220°C , total run time: 63.67 min. For the SPME extraction a manual fiber holder Supelco Inc. (Bellefonte, PA, USA) with a $50\ \mu\text{m}$ of DVB/CAR/PDMS fiber Supelco Inc. (Bellefonte, PA, USA) were used. After every analysis the fiber was conditioned in the GC inlet for 1 h at 270°C , according to manufacturer's instructions. Furfural was identified base on retention time and mass spectra of the MS spectral library data of the standard compound.

The method used for sugars content analysis in the liquid fraction was liquid-liquid extraction, followed by oximation and silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and, finally, analysis by GC-MS, according to our previous studies [19].

Results and discussion

The composition of wood consists of cellulose, hemicellulose and lignin components. Composition of wood was determined and it was: 46% cellulose, 24% hemicellulose and 28% lignin; the results are similar with other works reported [12, 20]. According to theory (UNCTAD/GATT 1979) the furfural can be produced from lignocellulosic materials at industrial level, if these contain 18-20% pentosans [21]. Generally, hydrolysis of hemicellulose is done using acids, alkali or organic solvents. In this study, autohydrolysis was used as pretreatment of wood for hemicellulose separation in liquid fraction. Autohydrolysis caused direct hydrolysis of hemicellulose in liquid fraction into sugars oligomers and monomers. In solid fraction are recovered cellulose and lignin due to extremely insolubility of cellulose and lignin in water at high temperature and pressure. Content of pentoses and hexoses was analyzed by GC-MS after derivatisation procedure. Figure 2 shows the gas chromatogram of carbohydrates obtained after autohydrolysis pretreatment of wood.

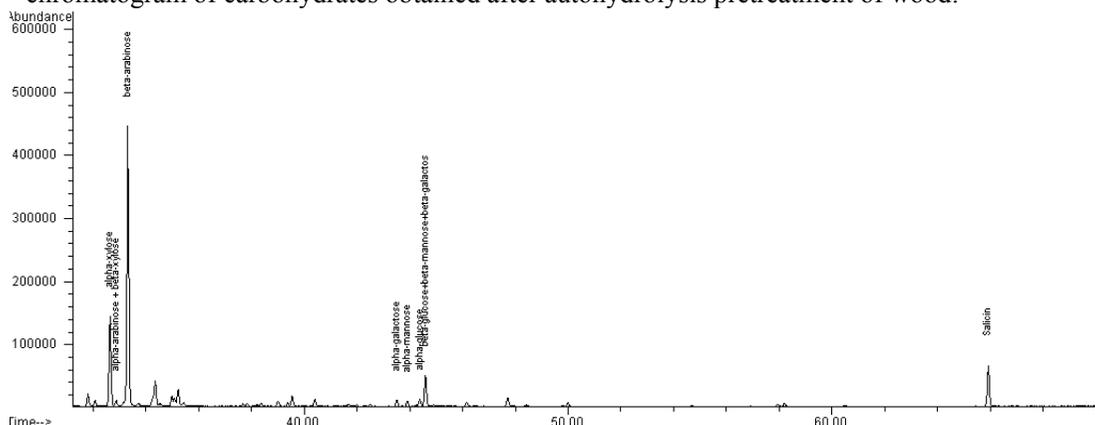


Figure 2. The GC-MS chromatogram of carbohydrates obtained after autohydrolysis pretreatment of wood

In hemicellulosic fraction was found a mixture of α and β isomers of each sugar (arabinose, xylose, glucose, mannose and galactose). The GC chromatogram shows that the predominant components of hemicellulose fraction are pentoses (β -arabinose is the easiest extractable sugar). Arabinose is the most susceptible to degradation, and can give furfural as secondary reaction byproduct.

The contents of pentoses and hexoses in hemicellulose fraction resulted after autohydrolysis pretreatment are shown in figure 3, for different pretreatment conditions.

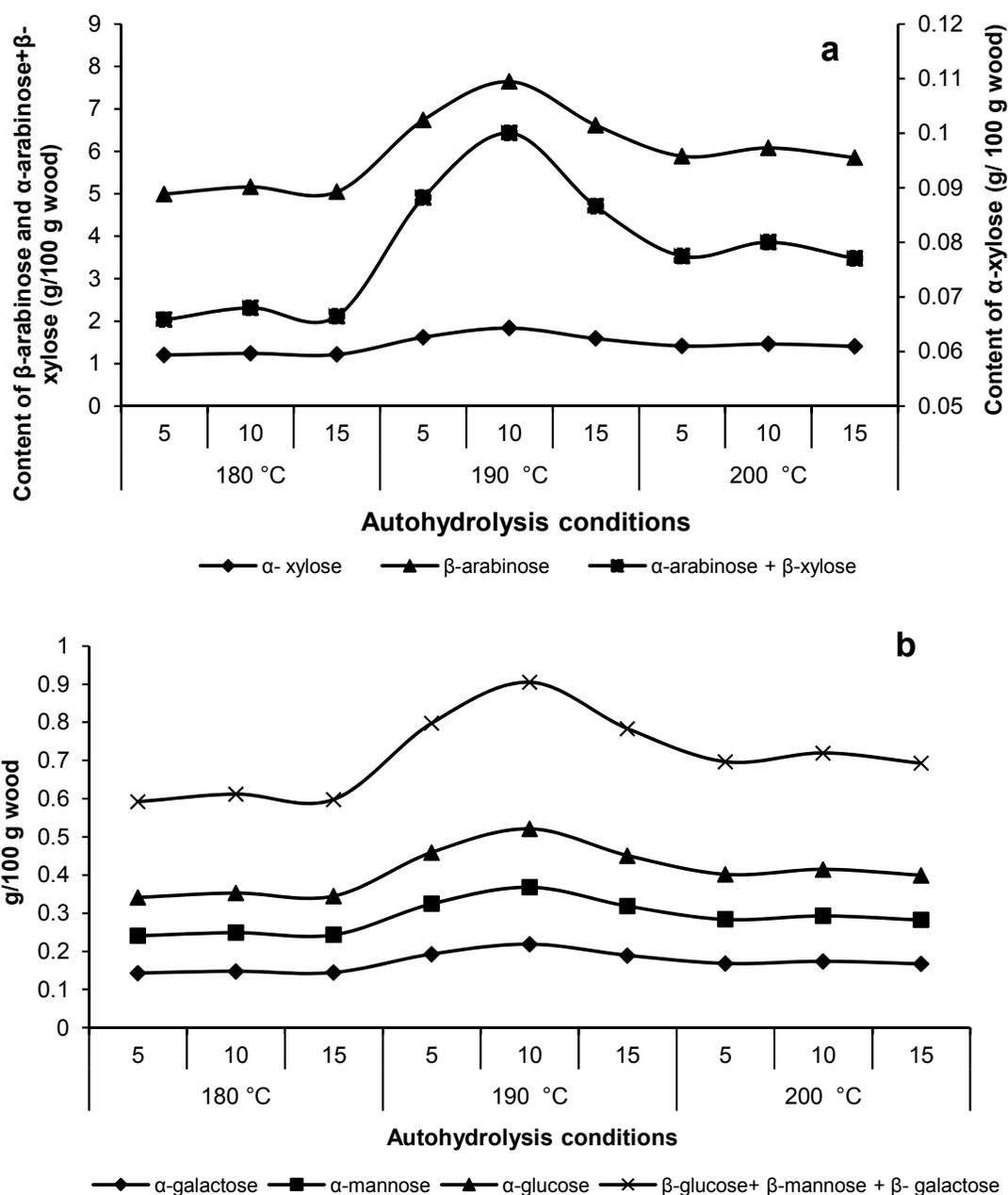


Fig. 3. Content of sugars after autohydrolysis pretreatment of wood: (a) pentoses isomers, and (b) hexoses isomers

Autohydrolysis method performs two functions: the function of pretreatment of wood separation and direct hydrolysis of existing pentoses into furfural. The efficiency of autohydrolysis pretreatment depends on temperature and reaction time. The quantities of sugars in hemicellulosic fraction were in the range 6.85 -10.48 g/100 g wood, function of autohydrolysis conditions. The highest concentration of pentoses (9.5 g/100 g wood) was obtained for autohydrolysis performed at 190°C for 10 min time of reaction. The lower

concentration of hexoses confirmed that cellulose was unaltered during autohydrolysis and the majority components of hemicellulose are pentoses.

The analysis of furfural from hemicellulosic fraction was done by two extraction methods: LLE and HS-SPME gas chromatography. Analysis of furfural from hemicellulosic fractions is influenced by several factors: method of extraction, temperature and extraction time. The two analytical methods above-described were compared from analytical performance point of view (the furfural content from wood hydrolysates). LLE extraction is one of the most commonly technique for extraction of many type of compounds [6]. Extraction of furfural after autohydrolysis by GC method was not reported in literature. Usually, furfural was analyzed by HPLC method [22]. Liquid-liquid extraction was made for furfural extraction from hemicellulose fraction, according to Morales et al. studies [23] for extraction of furfural from wine, with some modifications. Hemicelluloses matrixes are very complex matrices that contain oligosaccharide, monosaccharide, phenolic acids and inhibitory compounds. In recent years, different fibers with different adsorbent polymers, were applied such as: polydimethylsiloxane (PDMS) for extraction of nonpolar volatile; polyacrilat (PA) for semi-volatile polar compounds; divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), carboxen/polydimethylsiloxane (CAR/PDMS) and carbowax/polydimethylsiloxane (CW/PDMS) for aromatic compounds. These fibers are used for extraction of volatile and semi-volatile aromatic compounds. The commercial fiber DVB/CAR/PDMS with triple phase was a good choice for extraction of furfural from hemicellulosic fraction, due to the high efficiency in several analysis of volatile from food, wine, fruit juice, gin, etc. [22, 24].

SPME extraction method can be achieved in three ways: direct extraction of the sample in liquid, extraction in the headspace, and extraction with a protective membrane (fiber is separated from the sample through a selective membrane). The extraction technique used for extraction of furfural from hemicellulosic fraction is HS-SPME. The fibers could be protected against the adverse effects caused by non-volatile compounds present in the sample liquid, such as carbohydrates, using the HS-SPME technique.

The SPME extraction is influenced by the following factors: the polarity of fiber and compound, temperature, time and sample volume. Carboxen polymer has the property to retain the small molecules with high diffusion and the big molecule is retained in the outer layer of DVB phase [15]. A higher temperature of extraction improves the extraction of compounds with small volatility but, in the case of furfural, was found that a higher temperature increased the furfural degradation (the carbohydrates, esters and phenolic acids content increased with the temperature increasing). Taking into account all these considerations, 25°C was chosen as temperature for extraction of furfural. Samples were analyzed by both extraction procedures (LLE and HS-SPME).

For each method, the linearity, detection limits, quantification limits, recovery and reproducibility were determined. The linearity was studied using standard solution of furfural (working solution obtained by diluting stock solutions). The detection limits were calculated as lowest concentration that can be determined with an acceptable level of repeatability and fidelity, by consecutive dilutions. The quantification limits were calculated to the three times of limits of detection. Recovery studies were carried out by spiking hemicellulosic fraction resulted after autohydrolysis pretreatment methods with known amount of furfural. Quantification was made by external calibration. The analytical parameters for both extraction methods are summarized in Table 1.

Table 1. Linearity range, correlation coefficient, detection limits, quantification limits and recovery obtained for each method for the analysis of furfural from hemicellulose hydrolysates

Method	Linearity		Detection limit ($\mu\text{g ml}^{-1}$)	Quantification limit ($\mu\text{g ml}^{-1}$)	RSD (%) $n=5^a$	Recovery (%) (mean \pm SD, $n=5$) ^b
	Range ($\mu\text{g ml}^{-1}$)	R ²				
LLE	10–350	0.995	3.00	9.00	10.5	78 \pm 6.3
HS-SPME	0.1–35	0.999	0.03	0.09	7.3	95 \pm 5.9

^aRelative standard deviation^bRecovery of furfural of spiked solution (with standard deviation (SD) in parentheses)

Detection limit for HS-SPME is lower ($0.03 \mu\text{g ml}^{-1}$) comparatively with LLE method ($3.0 \mu\text{g ml}^{-1}$). The linearity domain is similar for the two methods.

The reproducibility was estimated as the relative standard deviation (RSD) for five extraction of hemicellulosic fraction. The RSD for LLE method was 10.5% and 7.3% for HS-SPME method, these values are acceptable.

In Table 2 are presented the comparisons between the extraction methods.

Table 2. Comparison between LLE and HS-SPME method

Parameters	LLE	HS-SPME
Recovery (%)	78	95
Volume of samples	10–30 ml	1–10 ml
Volume of solvents	10 ml	No use
Time (min)	50 min	160 min
Performing	Complex	Simple
To environment	More polluting	No polluting

HS-SPME extraction method has many advantages comparatively with LLE method: better recovery, lower volume of sample, no use chemical, and being in this way, environmental-friendly.

The GC chromatogram of the hemicellulosic fraction obtained after the HS-SPME extraction is shown in Figure 4. Retention time for furfural was 8.480 min (the furfural component of the hydrolysate was identified by the coincidence of the retention time and mass spectra of the standard solution).

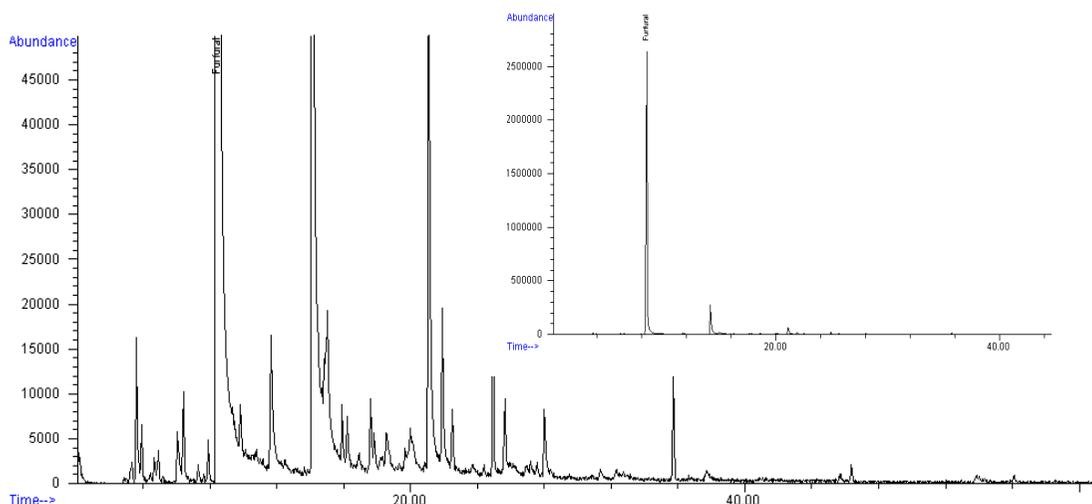
**Figure 4.** Gas chromatography chromatogram for furfural analysis from hemicellulosic fraction using HS-SPME extraction method (180°C for 10 min)

Figure 5 shows the furfural contents in hemicellulose fraction, function of reaction conditions.

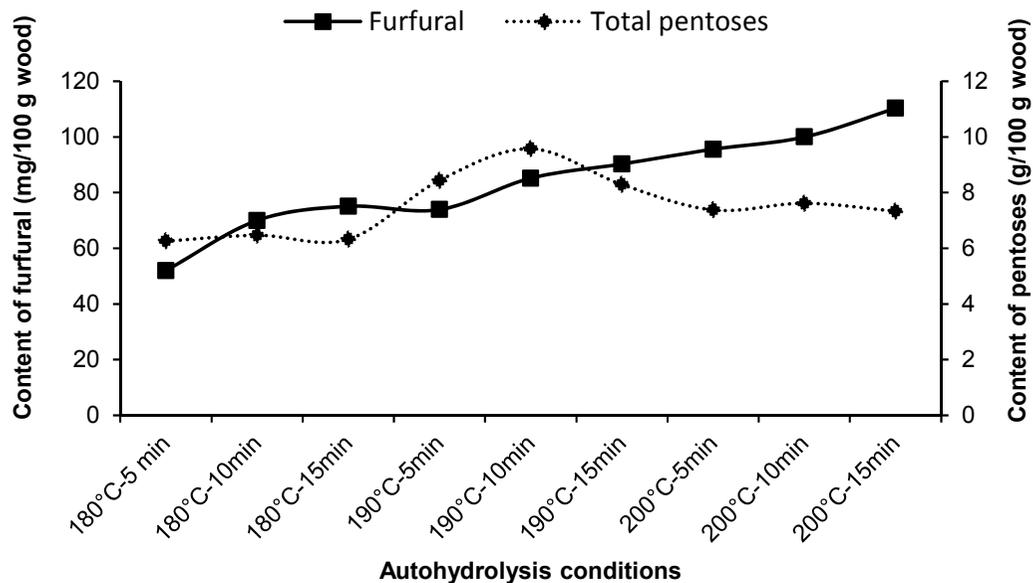


Figure 5. Furfural contents from hemicellulosic fraction obtained from wood

Content of furfural, one of the pentose degradation products, increased with temperature and reaction time increasing. Concentration of furfural obtained after autohydrolysis pretreatment of wood has a linear distribution, function of temperature and time. Furfural content in hemicellulosic fraction obtained from wood after autohydrolysis pretreatment is comparable with those obtained by other authors [11, 14, 25].

Conclusions

In this work, liquid-liquid and headspace solid phase microextraction methods, followed by GC-MS were successfully performed to determine the furfural compound in hemicellulose hydrolysate. Autohydrolysis pretreatment of wood was used for conversion of biomass into furfural. The obtained results demonstrate that HS-SPME has many advantages comparatively with LLE extraction: simple, single step extraction, solvent-free, powerful method. Increasing temperature and residence time increase the conversion of pentose into furfural. A temperature of 200°C and a residence time of 15 min give the high furfural content (100 mg/100 g wood).

Acknowledgments

This work was carried out with support from the UEFISCDI, PNII Program (Project HYCAT no. 43/2012).

References

- O. YEMIŞ, G. MAZZA Acid-catalyzed conversion of xylose, xylan and straw into furfural by microwave-assisted reaction. *Bioresour. Technol.*, 102, 7371-7378 (2011).
- O. AKPINAR, K. ERDOGAN, S. BOSTANCI, Production of xylooligosaccharides by controlled acid hydrolysis of lignocellulosic biomass. *Carbohydr. Res.*, 344, 660-666 (2009).

3. B.C. SAHA, Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.*, 30, 279-291 (2003).
4. P.S. NIGAM, A. SINGH, Production of liquid biofuel from renewable resources. *Prog. Energy Comb. Sci.*, 37, 52-68 (2011).
5. Y. SUN, J. CHENG, Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.*, 83, 1-11 (2002).
6. W. RIANSA-NGAWONG, P. PRASERTSAN, Optimization of furfural production from hemicellulose extracted from delignified palm pressed fiber using a two stage process. *Carbohydr. Res.*, 346, 103-110 (2011).
7. S. MAKISHIMA, M. MIZUNO, N. SATO, K. SHINJI, M. SUZUKI, K. NOZAKI, F. TAKAHASHI, T. KANDA, Y. AMANO, Development of continuous flow type hydrothermal reactor for hemicellulose fraction recovery from corncob. *Bioresour. Technol.*, 100, 2842-2848 (2009).
8. X. DU, M. QIAN, Quantification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone using solid-phase extraction and direct microvial insert thermal desorption gas chromatography-mass spectrometry. *J. Chromatogr. A.*, 1208, 197-201 (2009).
9. Z.Q. FAN, S.B. WANG, R.M. MU, X.R. WANG, S.X. LIU, X.L. YUAN, A simple, fast, solvent-free method for the determination of volatile compounds in *Magnolia grandiflora*. *J. Anal. Chem.*, 64, 289-294 (2009).
10. G. GARROTE, E. FALQUÉ, H. DOMÍNGUEZ, J.C. PARAJÓ, Autohydrolysis of agricultural residues: Study of reaction byproducts. *Bioresour. Technol.*, 98, 1951-1957 (2007).
11. S. CAPARRÓS, M.J. DÍAZ, J. ARIZA, F. LÓPEZ, L. JIMÉNEZ, New perspectives for *Paulownia fortunei* L. valorization of the autohydrolysis and pulping processes. *Bioresour. Technol.*, 99, 741-749 (2008).
12. J.Y. ZHU, X.J. PAN, Woody biomass pretreatment for cellulosic ethanol production: Technology and consumption evaluation. *Bioresour. Technol.*, 101, 4992-5002 (2010).
13. D. NABARLATZ, A. EBRINGEROVÁ, D. MONTANÉ, Autohydrolysis of agricultural by-products for the production of xylo-oligosaccharides. *Carb. Polym.*, 69, 20-28 (2007).
14. J.C. PARAJÓ, G. GARROTE, J.M. CRUZ, H. DOMINIQUEZ, Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials. *Trends in Food Science and Technology*, 15, 115-120 (2004).
15. S.M. ROCHA, M.A. COIMBRA, I. DELGADILLO, Occurrence of furfuraldehydes during the processing of *Quercus suber* L. cork. Simultaneous determination of furfural, 5-hydroxymethylfurfural and 5-methylfurfural and their relation with cork polysaccharides. *Carb. Polym.*, 56, 287-293 (2004).
16. B. QI, J. LUO, X. CHEN, Y. WAN, Separation of furfural from monosaccharides by nanofiltration. *Bioresour. Technol.*, 102, 7111-7118 (2011).
17. ANDUJAR-ORTIZ, M.V. MORENO-ARRIBAS, P.J. MARTÍN-ÁLVAREZ, M.A. POZO-BAYÓN, Analytical performance of three commonly used extraction methods for the gas chromatography-mass spectrometry analysis of wine volatile compounds. *J. Chromatogr. A.*, 1216, 7351-7357(2009).
18. G. GARROTE, H. DOMÍNGUEZ, J.C. PARAJÓ, Kinetic modelling of corncob autohydrolysis. *Process Biochem.*, 36, 571-578 (2001).
19. L. SENILA, A. GOG, M. SENILA, C. ROMAN, L. SILAGHI-DUMITRESCU, Analysis of carbohydrates obtained from wood by gas chromatography-mass spectrometry. *Rev. Chim.*, 62, 149-153 (2011).
20. A. WISELOGEL, S. TYSON, D. JOHNSON, (1996) *Biomass feedstock resources and composition*. In: Wyman, C. E. (Ed.), *Handbook on Bioethanol: Production and Utilization* Taylor & Francis, Washington, DC p.105-118.
21. UNCTAD/GATT, *Making and marketing furfural: added value for agro-industrial wastes*. Abstracts for Trade Information Services, International trade center, Geneva, 1997, p. 3.7.
22. E.M.S.M. GASPAR, A.F.F. LUCENA, Improved HPLC methodology for food control- furfurals and patulin as markers of quality. *Food Chem.*, 114, 1576-1582 (2009).
23. M.L. MORALES, B. BENITEZ, A.M. TRONCOSO, Accelerated aging of wine vinegars with oak chips: evaluation of wood flavor compounds. *Food Chem.*, 88, 305-315 (2004).
24. E.M.S.M. GASPAR, J.F. LOPEZ, Simple gas chromatographic method for furfural analysis. *J. Chromatogr.*, 1216, 2762-2767 (2009).
25. F. CARVALHEIRO, M.P. ESTEVES, J.C. PARAJÓ, H. PEREIRA, F.M. GÍRIO, Production of oligosaccharides by autohydrolysis of brewery's spent grain. *Bioresour. Technol.*, 91, 93-100 (2004).