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REVIEW

GC×GC in the Characterization of the Bio-Oil from Brazilian Biomass: A Review

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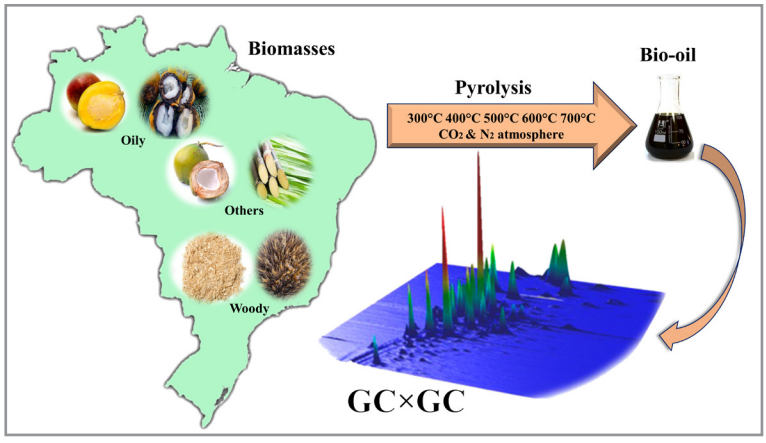

This study presents and discusses the state of the art of Two-Dimensional Comprehensive Gas Chromatography (GC×GC) developed in Brazil. GC×GC has been the focus of studies in Brazil since 2009, based on successful experiences in cooperation with researchers from Australia and Italy. The result of these researches led to the installation of many laboratories in Brazilian Universities and Research Centers, similar to others in foreign countries and the development of research, mostly involving applications of the technique to Brazilian matrices. In this review we present applications of GC×GC involving the pyrolysis of Brazilian agroindustrial residues, such as cane straw, sawdust, coconut fiber, fruit seeds, rice husks, spent coffee grounds, among others. The most used detection techniques for GC×GC have been mass spectrometry with fast quadrupole analyzer (GC×GC/qMS) and time of flight (GC×GC/TOFMS). These studies showed the possibility of identifying many organic compounds in the bio-oils produced, especially oxygenated ones such as phenols, ketones, acids and esters. Several studies suggest catalytic pyrolysis as a way to generate less oxygen-compounds directing the application of this bio-oil to the area of biofuels. However, the compounds found and their relative concentration, indicates that the best uses should be associated with the processing industry such as pharmaceuticals, chemicals, polymers and food.

Keywords: bio-oil, characterization, Brazilian biomasses, gas chromatography, GC×GC.

INTRODUCTION

Comprehensive two-dimensional gas chromatography (GC×GC) is a technique originally described in 1991 by Professor John B. Phillips and his student Zaiyou Liu [1]. Since then, this technique has been extensively applied to solve complex separation problems such as the large amount of analytes with

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structural similarities, analytes with large concentration differences between them and also the presence of unresolved complex mixture in 1D. In the last 30 years, GC×GC began to attract attention and their 3D (three-dimensional) chromatograms wake-up the interest of analytical chemists for these new way for analyzing and presenting the results related to complex mixtures [2].

Using GC×GC, the separation power of a gas chromatography system is optimized by coupling two columns with different polarities. Thus, the eluent from 1D (first dimension) column is conducted to the 2D, through a modulator, which segments and focuses the effluent from the first column to the second column [3].

Initially, the main reviews discussed the principles of the technique, the basic theory and the experimental set-up [1,3]. Next, different interfaces among columns became a key topic and the first few applications were reported. Most of these were in the field of petrochemical analysis [4]. In the past few years the main parameter studied were software, new columns and others novelties, indicating that great steps for-ward still have to be made (e.g., in detection, analyte identification and quantification and, specifically, applications). Today, besides petrochemical analysis, areas such as food, air and environmental analysis are detailed studied by GC×GC [2,3,5-7].

Many researches in GC×GC were developed in Brazil, based on successful experiences in scientific exchanges with researchers from Australia and Italy between 2005 and 2007. The first works were related to characterization of oil and derivatives. Then, due to the characteristics of Brazil in terms of environment, energy and biodiversity, this application evolved and the first works involving pesticides, plants and biomass began to be produced. As of 2007, with the installation of some equipment in the Brazilian laboratories of the Universities involved, these researches gained more impact and currently several modern GC×GC systems can be found in the Brazilian Institutions, devoted to a wide variety of researches, from natural products, drugs, pesticides, biomass and biofuels [8-14].

In this review it is covered the literature from 2009 to 2020 on the applications of GC×GC involving the pyrolysis of Brazilian agribusiness residues, such as cane straw, sawdust, coconut fiber, fruit seeds, rice husks, spent coffee grounds, among others.

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GC×GC)

Characteristics

Comprehensive two-dimensional gas chromatography (GC×GC) developed in 1991 by Liu and Phillips has several advantages when compared with one-dimensional gas chromatography. The analyte separation mechanism is performed by two interconnected columns, with a transfer system (modulator) located between them [1], as seen in Figure 1. The modulation process is the main stage of the system, which is responsible for trapping, focusing and introducing fractions of the effluent from the column of the first-dimension (1D) into the second-dimension column (2D). This can be achieved through thermal or flow modulators [2,3,5].

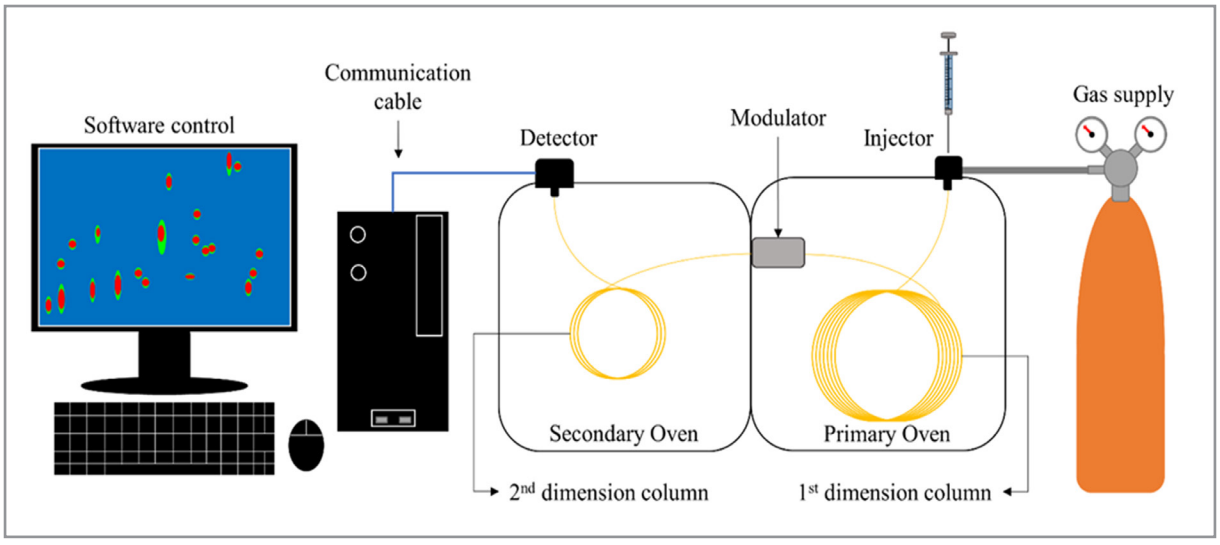

Figure 1. Comprehensive two-dimensional gas chromatography system: injector; 1st dimension column; 2nd dimension column; modulator; detector. (Liu, Zaiyou; Phillips, John B. Comprehensive Two-Dimensional Gas Chromatography using an On-Column Thermal Modulator Interface. *Journal of Chromatographic Science*, 1991, Vol 29, Issue 6, pages 227–231, by permission of Oxford University Press.) [1].

Considering that the peak capacity of a column is the maximum number of peaks separable by it, it can be assumed that the peak capacity of 1D is n_1 peaks, while that of 2D is n_2 peaks. Thus, it can be said that the peak capacity of the GC×GC will be n_1 x n_2 peaks, since the entire sample is subjected to separation in both columns. Comparatively, the peak capacity of the two-dimensional gas chromatography (GC-GC, only a part of the analytes from ^{1}D are also separated in ^{2}D) will be $n_1 + n_2$, since only a fraction of the sample is subjected to separation in 2D [15]. Among these advantages, we can highlight a higher resolution, peak capacity, selectivity and structured elution of sample components in the chromatogram according to their physicochemical properties [6,7].

The first column is usually of low polarity, with analyte separation based on the boiling point. Each modulated fraction is then subjected to a fast GC analysis on the second dimension, generally on a polar column: analytes are resolved on the basis of specific polarity-based characteristics. It is important that the system presents orthogonality, so that the interaction mechanism of 1D is as different as possible from the mechanism used in 2D [6].

The time in which the modulator samples the effluent from 1D and directs it to 2D is a crucial factor for the functioning of the GC×GC, as it directly influences the quality of the chromatogram to be obtained [6]. The sampling sequence followed by injection must be precisely defined and repeated throughout the chromatographic analysis. This time interval is called the modulation period. Ideally, the separation in 2D should occur before the injection of the subsequent chromatographic fraction to minimize the occurrence of peak wraparound [6,7,16].

For bio-oils, the modulation step is important mainly for two reasons: isolating the chromatographic bands coming from the ^{1}D, and re-concentrating these bands to quickly reinject in the ^{2}D. Due to the fact that bio-oils usually present analytes in very different concentration ranges, this re-concentration of the chromatographic band in the modulator facilitates the detection/identification of compounds in low concentrations, which, eventually, could not be detected in 1D.

GC×GC allows a separation of analytes in two-dimensional space according to the chemical classes present in the sample. The orthogonality of the columns used allows this distinction between chemical classes. Some works demonstrate this characteristic, as in the work by von Mühlen et al. [12] shown in Figure 2. The chromatogram in Figure 2 refer to the analysis of *Eucalyptus dunnii* essential oil. The authors managed to divide the chromatograms into distinct regions of: (A) Linear alcohols; (B) aldehydes;

(C) acetates; (D) monoterpenic hydrocarbons; (E) monoterpenic alcohols; (F) monoterpenic acetates; (G) sesquiterpenic hydrocarbons; (H) oxygenated sesquiterpenes.

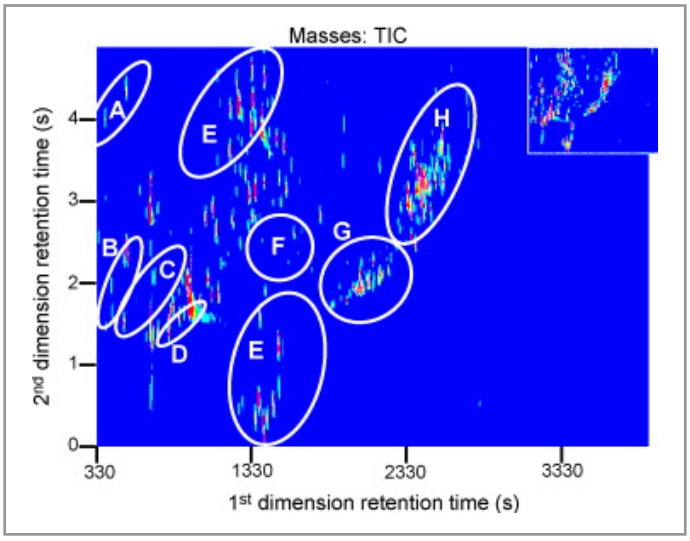

Figure 2. GC×GC/TOFMS total ion current chromatogram (TIC) data colour plot of *E. dunnii* essential oil, showing the distribution of classes of compounds in different regions of the chromatographic space, using a non-polar×polar column set. (A) Linear alcohols; (B) aldehydes; (C) acetates; (D) monoterpenic hydrocarbons; (E) monoterpenic alcohols; (F) monoterpenic acetates; (G) sesquiterpenic hydrocarbons; (H) oxygenated sesquiterpenes. ("Reprinted from *Journal of Chromatography A*, Vol 1200, Issue 1. Authors: Carin von Mühlen, Claudia Alcaraz Zini, Elina Bastos Caramão, Philip J. Marriott. Title: Comparative study of *Eucalyptus dunnii* volatile oil composition using retention indices and comprehensive two-dimensional gas chromatography coupled to time-of-flight and quadrupole masss pectrometry, pages 34–42. Copyright 2008, with permission from Elsevier.) [12]

Several detectors can be coupled to GC×GC, these must have as main characteristics a high acquisition rate, low internal volumes and low time constants [17]. The first detector with high acquisition rates used in GC×GC was the flame ionization detectors (FID) that present acquisition rates from 50 to 200 Hz [15].

Later, mass spectrometry detectors (MS) began to be introduced to this technique; the time-of-flight mass spectrometry detector (TOFMS), allows the collection of up to 500 mass spectra per second. However, the fast-qMS analyzer has consolidated its application in the field of GC×GC due to the development of systems that allow acquisition rates around 50 Hz [12]. The combination of MS with GC×GC provide three analytical dimensions, which stands out as de most important tools for the characterization and identification of complex samples [18].

GC×GC/MS can be used to conduct large scale studies, giving full access to its high-resolution power for targeted and mostly untargeted screening. The current challenges in this area are localized on the data management side, where powerful chemometric tools are required to unlock GC×GC/MS full potential [19].

Comprehensive two-dimensional gas chromatography (GC×GC) has generated a major impact in separation science. In the past 30 years, around 3700 papers were found in the scientific literature through a search on the Scopus Platform using the term "Comprehensive Two-dimensional Gas Chromatography".

History in Brazil

The first research developed in Brazil using GC×GC was in 2009, in which the analysis of organic compounds from water-in-crude oil emulsions separated by microwave heating using comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry [20] was used. The GC×GC/TOFMS technique proved to be extremely important for the separation and identification of different classes of

compounds. This research represents a milestone in Latin America for separation chemistry, as it was carried in the first GC×GC system in Latin America set up at Universidade Federal do Rio Grande do Sul, Brazil.

Since then, several studies have been developed with different types of matrices. Figure 3 was obtained from a survey of articles in the Scopus database, using the term "Comprehensive two-dimensional gas chromatography" and limiting it to research developed in Brazil. The keywords were processed considering the type of matrices analyzed with at least 3 occurrences, using the VOSviewer 1.6.13 bibliometric software.

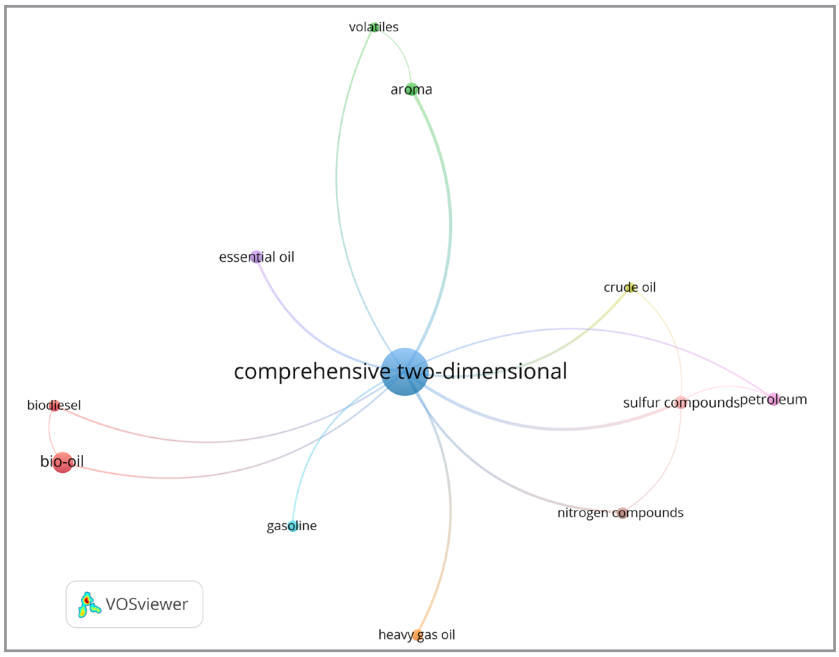

Figure 3. Bibliometric analysis of the most frequently cited keywords related to the type of matrix analyzed by GC×GC in Brazil using the Scopus database.

As shown in Figure 3, several complex matrices have already been analyzed using GC×GC in Brazil, where the largest number of articles found reports their use for bio-oil analysis. This fact can be explained due to the great complexity of this type of matrix, and also by the fact that Brazil presents a large investment in this sector. Brazil has been investing in renewable energy, mainly aiming at the use of agricultural and agro-industrial wastes. For this reason, bio-oil matrices were chosen for the development of this review.

BIO-OIL

Characteristics

The bio-oil, also known as pyrolysis oil, crude bio-oil, pyrolytic tar, wood liquid, wood oil, smoke condensate or wood distillate is the liquid product generated from the pyrolysis of lignocellulosic material. It has a dark brown color, almost black, and a characteristic odor [21,22]. Its composition depends on numerous variables, from the source of the raw material (biomass), through the instrumental conditions of the pyrolytic process (type of reactor, heating rate, final temperature, etc.) to the use of catalysts and the use of upgrading processes (chemical extraction, refining, hydrocracking, hydrodeoxygenation, steam reforming, esterification, emulsification, etc.) [23,24].

For proposing a better use of bio-oil, it is necessary to know its physico-chemical properties and its chemical composition. Several physical and chemical methods for the characterization and analysis of bio-oil have been used. This applied to properties such as ash and water content, elementary analysis, total solid content, heating value, density, viscosity, acidity and solubility in different solvents, in addition, of course, to their chemical composition. Table I lists the main characteristics and physicochemical properties of bio-oil [23,25–30].

Table I. Elementary composition and physicochemical properties of bio-oil

Properties	Characteristics	Reasons
Appearances	Dark brown to black	Bio-oil chemical composition [23]
Smell	Smoke odor	Aldehydes and acids of low molecular weight [23]
Density	1.2 kg L^{-1} (greater than fossil fuels)	High humidity and presence of high molecular weight molecules [23,25]
Viscosity	40 to 1000 centistokes (cSt)	Composition of biomass, water content and light product content [25]
Heating value	Lower than that of fossil fuels; 16-20 MJ kg^{-1}	High oxygen contente [23,25,26]
Miscibility	Miscible in polar solvents, but totally immiscible in oil	Polar nature [23]
Aging	Increased viscosity, decreased volatility, phase separation and resin deposition	Complex structures and acid pH [23]
Water content	15-35%	Residual water from biomass and parallel reactions [23,26,27]
Carbon content	50-64%	Biomass composition and thermal conversion process [23,28]
Hydrogen content	5-7%	Biomass composition and thermal conversion process [23,28]
Oxygen content	15-40%	Biomass composition and thermal conversion process [23,28]
Ash content	0.01-0.6%	Biomass composition [23,29,30]
Acidity	pH bellow 4	Biomass composition and thermal conversion process [23,25]

Chemical characterization

The chemical characterization of bio-oils is important not only to propose the best use of these oils, but also to determine the presence of possible harmful compounds that may be formed during pyrolysis process. Thus, one can evaluate the possibility of bio-oil being a renewable fuel source or used as a starting material for obtaining chemicals. For example, a bio-oil with a higher percentage of phenolic compounds

can be applied as a substitute for fossil phenols in phenolic resins for the production of chemicals. A bio-oil that presents a significant amount of long-chain carboxylic acids and hydrocarbons is more applicable for use as liquid fuel after an upgrading process [30].

The analytical techniques frequently used for bio-oil analysis are chromatographic methods, such as Gas Chromatography (GC) and Liquid Chromatography (LC) (as they allow the separation and identification of organic compounds in complex matrices) and spectroscopy methods such as Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). These techniques are generally the most used due to their high precision and sensitivity [31]. The GC×GC, on the other hand, has been proved to be more efficient regard about to sensitivity, resolution and peak capacity, when compared to one-dimensional gas chromatography.

A representative list of some compounds present in bio-oils and their respective chemical classes are shown in Figure 4. The compounds and the amount of each analyte present in the bio-oil depend on several factors such as the type and composition of the biomass, pyrolysis process, biomass storage, among others [32].

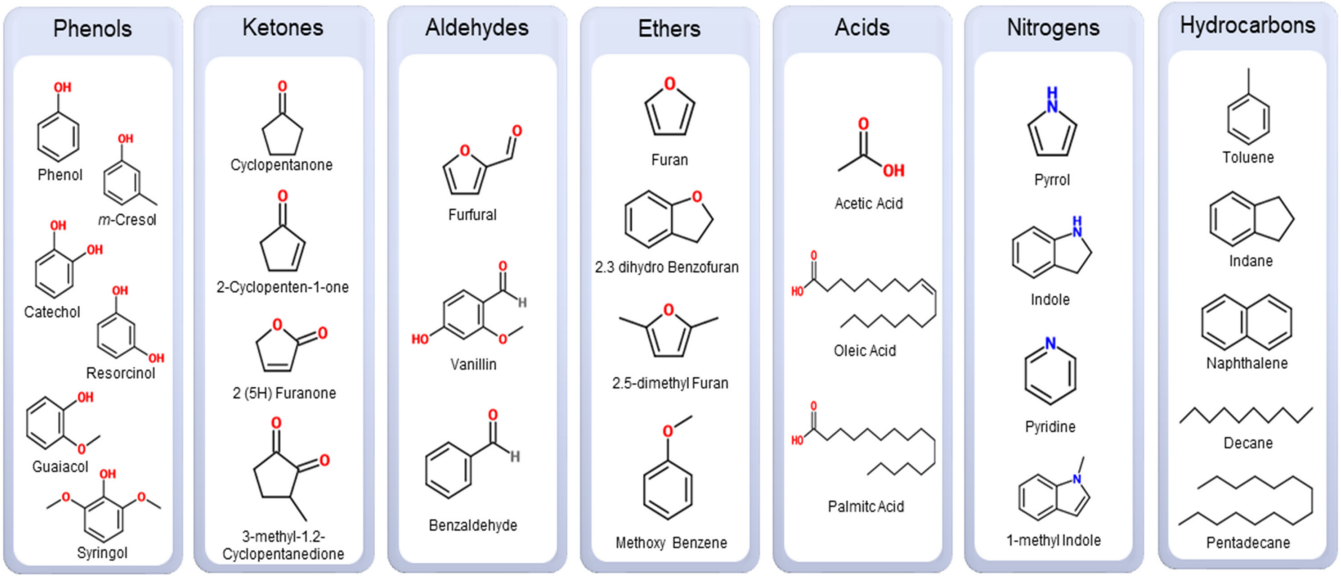

Figure 4. Main compounds found in bio-oils and their respective chemical classes.

GC×GC FOR BIO-OILS OF THE BRAZILIAN BIOMASSES

In Brazil, there are several studies using GC×GC to characterize bio-oils from lignocellulosic biomass, and it is possible to note that the main these bio-oils are produced from biomass from agro-industrial residues, not competing with food production. The research developed for the analysis of bio-oils derived from the pyrolysis of different Brazilian biomasses, were subdivided, in this review, into woody, oily and others biomasses.

In these studies, the injection modes used were split or split/splitless, with 1 µL of sample being used in most cases. Conventional sets of columns were used in most of the analysis: phases with 5% phenyl and 95% poly(dimethylsiloxane) in the first dimension, 30-60 length (m) x 0.18-0.25 i.d. (mm) x 0.10-0.25 thickness (µm), and 50% phenyl and 50% poly(dimethylsiloxane) in the second dimension, 1.10-2.50 length (m) x 0.10-0.25 i.d. (mm) x 0.10-0.25 thickness (µm). Three different types of detectors were coupled to the GC×GC: flame ionization detector (FID), quadrupole mass spectrometry (q/MS) e time-of-flight mass spectrometry (TOFMS). And finally, hydrogen (for GC×GC/FID) and helium (for GC×GC/qMS and TOFMS) were used as carrier gas at 0.70-1.20 mL min^{-1}.

The qualitative characterization of the compounds has been carried out using the chromatographic techniques associated with the Linear Temperature Programming Retention Index (LTPRI), where a standard mixture of linear hydrocarbons is analyzed under the same conditions as the samples [33] and compared with standard compounds and the literature (webbook.nist.gov). The use of retention index identification is tentative identification. For positive identification, it is necessary to use reference standards.

The mass spectra of each analyte are frequently used, facilitating the compounds identification by comparing with the mass spectra of commercial libraries (NIST or Wiley). Some studies also evaluate the compounds semi-quantitatively, using the relative percentage area of the chromatographic peaks and quantitatively, using standards and calibration curves. Table II presents a summary of the studies that have used Brazilian biomass for bio-oil production, and characterization by GC×GC detailing the analysis settings, as well as the main classes of compounds found.

Table II. Researches performed with Brazilian biomass for analysis by GC×GC

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Woody	*Eucalyptus sawdust*	—	DB-5 (50x0.25x0.25)	DB-17 (2.15x0.18x0.18)	60°C (0.20 min) – 3°C min^{-1} – 330°C (20 min)	10 °C above the ^{1}D	He; 1.20	TOFMS	Phenols and ketones	34
	Sawdust from forest timber	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.15x0.18x0.18)	40°C – 3°C min^{-1} – 120°C – 2°C min^{-1} – 200°C – 10°C min^{-1} – 280°C (5 min)	The same as ^{1}D	He; 0.91	q/MS	Phenols and ketones	35
	Eucalyptus sp and Picea abies	Fast and Catalytic	DB-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	60°C (3 min) – 3°C min^{-1} – 240°C (10 min)	10 °C above the ^{1}D	-	TOFMS	Phenols and ketones (fast); Polyaromatic hydrocarbons (catalytic)	36
	Pinewood	Catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He	TOFMS	Ketones and hydrocarbons	37
	Pinewood	Fast, Catalytic and hydrodeoxygenation	DB-5 (30x0.25x0.25)	BPX-50 (1.50x 0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C (3 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Sugars (fast); Aromatic hydrocarbons (catalytic); Alcohols (hydrogeoxygenation)	38
	Palm fruit bunch and pinewood	Flash	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (15 min) – 4°C min^{-1} – 330°C	20 °C above the ^{1}D	He; 1.00	TOFMS	Ketones, cyclopentenones, furanones, furans and phenols	39; 40
	Pinewood	Fast and catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x 0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Lactones, Ketones and acids (fast); Aromatic hydrocarbons (catalytic)	41

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Oily	*Spent coffee grounds*	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.50x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 280°C	5 °C above the ^{1}D	He; 1.00	TOFMS	Hydrocarbons, nitrogen compounds and fatty acids	42
	Silverskin	Fast	OV-5 (60x0.25x0.10)	DB-17MS (2.50x 0.18x0.18)	40°C (5 min) – 5°C min^{-1} – 300°C (20 min)	The same as ^{1}D	He; 0.91	q/MS	Phenols and nitrogen compounds	43
	Bark of acuri and endocarp of baru	Fast	DB-5 (60x0.25x0.10)	DB-17MS (2.50x0.18x0.18)	50°C (5 min) – 4°C min^{-1} – 280°C (8 min)	10 °C above the ^{1}D	-	TOFMS	Phenols and ketones (bark of acuri); Hydrocarbons and phenols (endocarp of baru)	44
	Peach pit	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.84x0.18x0.18)	40°C (5 min) – 5°C min^{-1} – 280°C (10 min)	20 °C above the ^{1}D	-	TOFMS	Phenols and ketones	45
	Castor seed cake	Slow	DB-5 (30x0.25x0.25)	BPX-50 (1.50x 0.10x0.10)	35°C (15min) – 4°C min^{-1} – 330°C	20 °C above the ^{1}D	He; 1.00	TOFMS	Nitrogenous compounds and phenols	32
	Tabacco seeds	Fast	DB-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	40°C (2 min) – 4°C min^{-1} – 280°C (3 min)	The same as ^{1}D	He; 0.89	q/MS	Phenols and hydrocarbons	46
	Mango seed waste	Fast	DB-5 (60x0.25x0.25)	DB-17MS (1.20x0.18x0.18)	50°C (4 min) – 4°C min^{-1} – 280°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols and ketones	47
	Crambe seeds	Slow	DB-17 e DB-5	BPX-50 e DB-5	35°C (6 min) – 3°C min^{-1} – 330°C	5 and 10 °C above the ^{1}D	He; 1.00	TOFMS	Hydrocarbons (bio-oil); Amides and carboxylic acids (Aqueous phase)	48
	Crambe seeds	Fast	DB-5MS (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	50°C (1 min) – 4°C min^{-1} – 280°C (10 min)	5 °C above the ^{1}D	He; 1.00	TOFMS	Fatty acids, hydrocarbons and phenols	49
	Soursop seed cake and bocaiuva seed cake	Slow	DB-5 (30x0.25x0.25)	DB-17 (1.20x0.10x0.10)	35°C (15 min) – 3°C min^{-1} – 330°C	5 °C above the ^{1}D	He; 1.00	TOFMS	Carboxylic acids and amides (soursop seed cake); Hydrocarbons and phenolic derivatives (bocaiuva seed cake)	50

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Others	*Sugar cane straw*	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.20x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 315°C (10 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	51
	Sugar cane straw	Fast	OV-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 280°C (25 min)	The same as ^{1}D	He; 0.89	q/MS	Phenols, ketones and aldehydes	52
	Sugar cane straw	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	100°C (0.2 min) – 2°C min^{-1} – 210°C (3 min)	20 °C above the ^{1}D	-	TOFMS	Phenols, aldehydes and ketones	53
	Sugar cane bagasse	Catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He	TOFMS	Ketones and carboxylic acids	37
	Sugar cane bagasse and straw	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.15x0.18x0.18)	40°C – 5°C min^{-1} – 300°C (20 min)	The same as ^{1}D	He; 1.00	q/MS	Phenols and furans	54
	Sugar cane bagasse	Fast and catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x 0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Lactones, Ketones and acids (fast); Aromatic hydrocarbons (catalytic)	41
	Coconut fiber	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 210°C (3 min)	20 °C above the ^{1}D	He; 1.00	TOFMS	Phenols and aldehydes	55
	Coconut fiber	Fast	DB-5 (30x0.25x0.25) DB-5 (10x0.18x 0.25)	DB-17 (1.25x0.18x0.18) DB-17 (1.10x0.18x0.18)	45°C – 3°C min^{-1} – 260°C 45°C – 10, 15 and 20°C min^{-1} – 260°C	15 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones, aldehydes and acids	56
	Coconut fiber	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.10x0.18x0.18)	45°C – 5°C min^{-1} – 290°C (10 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	57
	Coconut fiber	Fast	DB-5 (10x0.18x0.25)	DB-17 (1.10x0.18x0.18)	45°C (2 min) – 15°C min^{-1} – 260°C (2.5 min)	15 °C above the 1D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	58
	Orange bagasse	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.30x0.25x0.25)	—	—	H; 1.00 and He; 0.70	FID and TOFMS	Acids, aldehydes, alcohols and ketones	59

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Others	*Digester residue and wastewater treatment sludge*	—	DB-5 (50x0.25x0.25)	DB-17 (2.15x0.18x0.18)	60°C (0.20 min) – 3°C min^{-1} – 330°C (20 min)	10 °C above the ^{1}D	He; 1.20	TOFMS	Phenols and Ketones (both biomass); Nitrogen compounds and alcohols (waster treatment sludge)	34
	Rice husk	Intermediate	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 280°C	The same as ^{1}D	He; 1.00	q/MS	Phenols and ketones	60
	Rice husk	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1,84x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 315°C	10 °C above the ^{1}D	—	TOFMS	Phenols and ketones	45
	Rice husk	Intermediate	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 280°C	The same as ^{1}D	He; 1.00	q/MS	Phenols and ketones	61
	Endocarp, bark and fiber of bocaiúva	Fast	DB-5 (60x0.25x0.10)	DB-17MS (2.15x0.18x 0.18)	50°C (5 min) – 4°C min^{-1} – 280°C (8 min)	10 °C above the ^{1}D	—	TOFMS	Phenols (endocarp and bark); Hydrocarbons (fiber)	62

Biomass used for bio-oil production and analysis by GC×GC are of the most several types. To facilitate the discussion of literature data, it was classified into 3 types: woody biomass, which corresponds to biomass rich in lignocellulosic materials; oily biomasses that correspond in general to seeds and nuts; and, another biomass, which corresponds to biomass not previously classified.

GC×GC applied to woody biomass bio-oils

Woody biomasses are rich in lignocellulosic materials and the goal of their study was originally for bio-fuels as alternative to fossil fuels. However, the bio-oil produced by pyrolysis of these material, as demonstrated by chromatographic analysis, have many oxygenated compounds which are not indicated for this purpose. Now, woody bio-oils are mainly studied for producing substances with high added value for the chemical industry. Faccini et al. [34], optimized the pyrolysis process (using different temperatures) with residues of Eucalyptus sawdust. The bio-oil from this process has undergone a qualitative and semi-quantitative characterization by GC×GC/TOFMS. The total number of compounds tentatively identified in the bio-oil was 146, which corresponded to a total detected area of 97%. Phenols and ketones were the predominant chemical classes. Despite the great analytical capacity to separate compounds from the chromatographic technique used in this research, some problems of co-elution of compounds could be observed in the analyses. However, mass spectral deconvolution, offered by the GC×GC/TOFMS software (CHROMATOF), played an important role to solve this analytical problem because it provided a separation between two or more compounds through differences in their mass spectra and retention times, once TOFMS assures constant ion ratios along the chromatographic peak.

Schneider et al. [35] studied the most polar fractions of sawdust from forest timber bio-oil produced by fast pyrolysis and characterized by GC×GC/qMS. The use of the fast-quadrupole as a mass analyzer represents a great impulse in the two-dimensional analysis, because this detector is robust, simple, reproductive, sensitive and cheaper if compared to time-of-flight mass detectors, which is the most used for complex samples. From this analysis, 130 compounds were identified (phenols, ethers, ketones, aldehydes, acids, alcohols and aromatic hydrocarbons). From these compounds, 57 were confirmed by LTPRI, corresponding to 43.8% of the total identified. The relative concentration (semi-quantitative) was expressed in relation to the peak volume (volume of the three-dimensional peak of each compound divided by the total peak volume x 100). The results showed phenols as the major class of this bio-oil, with 60% of the total volume, followed by ketones, with 25%. The 4-methyl-1,2-benzenediol (12.1%), 1,2-benzenediol (11.1%), C2-benzenodiol (7.1%) and phenol (4.8%) were the major compounds.

In the research described by Torri et al. [36], bio-oil fractions from fast and catalytic pyrolysis of forest residues (Eucalyptus sp. and Pices abies) were analyzed by GC×GC/TOFMS. The non-catalytic bio-oils presented, mainly, phenols, ketones and aldehydes, while the catalytic ones presented polyaromatic hydrocarbons, phenols and ketones. In this case, the use of the catalyst (ZSM-5, SiO_2/Al_2O_3 ratio of 140) favored the production of polyaromatic hydrocarbons.

Mendes et al. [37], carried out catalytic pyrolysis of pinewood and the bio-oil obtained was characterized by GC×GC/TOFMS. The ketones and hydrocarbons were the predominant chemical classes. The use of the ZSM-5 catalyst was mainly responsible for the significant increase in the content of aromatic hydrocarbons in bio-oil, mainly monoaromatics, such as the toluene and C2-benzene isomers (xylenes and ethylbenzene). These compounds are used as petrochemical intermediates with high added value for the chemical industry. In addition, the ZSM-5 reduced the number of oxygenated compounds such as phenols.

In the research done by Silva et al. [38], the authors performed a characterization (semi-quantitative and quantitative) by GC×GC/TOFMS of pinewood bio-oil from three pyrolytic processes: real thermal(PWT), catalytic process(CP) and hydrodeoxygenation process(HDO). The chromatographic method presented in this article proved to be suitable for the quantification of hydrocarbons and O-containing compounds in real samples of bio-oil with excellent accuracy and precision. The results obtained by the semi-quantitative analysis allowed a preliminary analysis by comparing the distribution of classes, where sugars, aromatic

hydrocarbons and alcohols appeared as the most abundant in PWT, CP and HDO bio-oils from pinewood, respectively. The quantitative evaluation allowed to obtain an individual concentration of target compounds. Among these, the 2(3H)-Furanone, dihydro-3-methyl had a higher concentration in the HDO bio-oil, while in the other samples (PWT and CP) the concentration were more evenly distributed in aromatic compounds.

In the studies developed by Tessarolo et al. [39,40], the authors obtained bio-oils from empty palm fruit bunch and pine wood chips from flash pyrolysis and characterized the fractions by GC/MS and GC×GC/TOFMS. The higher chromatographic resolution and sensitivity of the two-dimensional technique and the use of a detector with a higher data acquisition rate (TOFMS) allowed for better separation and greater identification (four to seven times more) of compounds in both samples, solving problems of co-elution found in the monodimensional analysis. The results of the two-dimensional analyses indicated that a large number of peaks were detected (631 and 857, respectively, for empty palm fruit bunch and pine wood chips bio-oils) and the main classes of compounds in both bio-oil samples were: ketones, cyclopentenones, furanones, furans, phenols and sugars. In addition, esters, aldehydes and pyridines were found for samples obtained from empty palm fruit bunch, while alcohols and cyclopentadiones were found in samples prepared from pine wood chips, indicating different composition profiles due to biomass source.

Tessarolo et al. [41] also submitted pine wood to the thermal and catalytic pyrolysis process using ZSM-5. The bio-oils obtained were characterized by GC×GC/TOFMS. The classes identified were lactones, cyclic ketones, acids, aldehydes, phenols and aromatic hydrocarbons. The use of the ZSM-5 catalyst promoted deoxygenation, reducing the content of oxygenated compounds, such as acids, and increasing the production of aromatic hydrocarbons, with alkyl-benzenes being the main components.

GC×GC applied to oily biomass bio-oils

Brazil is a great agricultural producer and the problem of the destination of agro-industrial waste has been explored in recent decades. Among these discharges stand out oily biomasses, such as seeds from agricultural productions, besides waste of important crops such as crambe, bocaiuva, coffee, soursop, tobacco, among others.

Coffee is an important agricultural product, being one of the most consumed beverages in the world and Brazil leads its worldwide production. Primaz et al. [42] used spent coffee grounds as biomass in the pyrolysis to obtain bio-oil. The characterization (qualitative and semi-quantitative) of the bio-oil was carried out by GC×GC/TOFMS. It was tentatively identified 190 compounds, belonging to the classes of hydrocarbons, nitrogen compounds and oxygen compounds, including ketones and phenols, in addition to oily compounds such as fatty acids and esters. Due to the high level of fats present in this biomass, the esters and fatty acid classes were found significantly, with the major compound being palmitic acid (19%). In this research, dispersion graphics were used to analyze the spatial distribution of the identified components, built from the retention times of the first and second dimensions. This distribution was made according to the molecular weight, and the number of substituents and branches, proving to be an excellent tool on qualitative analysis.

Polidoro et al. [43] submitted silverskin, a derived by-product from the coffee roasting process, to the pyrolysis process. In this research, the pyrolysis process was optimized using the response surface methodology and the bio-oil produced under the optimized conditions was analyzed by GC×GC/qMS. The use of this technique allowed the identification of 228 compounds, where the main chemical class, in terms of percentage of peak volume, was phenols (20.76%), followed by nitrogen compounds (18.51%). In this research, the use of dispersion graphics was also explored, ordering the chemical classes identified in the bio-oil according to molecular weight and polarity. The use of the GC×GC allowed to verify the presence of several groups of analytes with similar retention time in the first dimension, which would imply co-elutions in the use of the conventional GC.

The bio-oil of the acuri bark and baru endocarp residues were studied by Cardoso et al. [44] using GC×GC/TOFMS. In the qualitative analysis, 113 compounds were tentatively identified in the acuri bark

bio-oil, with phenols and ketones as the main chemical classes and guaiacol as the major compound, with 18.67% of the total area. For the baru endocarp residue bio-oil, 71 compounds were identified, with hydrocarbons and phenols being the main chemical classes and toluene as the major compound, contributing with 33.19% of the total area of the chromatogram.

The peach core was used as biomass for studies by Moraes et al. [45]. The pyrolysis parameters were optimized for another biomass (rice rusk) and the best conditions were applied for the production of peach stone bio-oil. This bio-oil was characterized qualitatively and semi-quantitatively using the GC×GC/TOFMS technique and 223 compounds classified as phenols and ketones (majority classes), acids, ethers and aldehydes were identified. The major compound found in this sample was furfural, with 8.82% of area.

Silva et al. [32] submitted the residual castor seed cake (after oil extraction process) to slow pyrolysis, obtaining a bio-oil yield of 22.3%. The chromatographic characterization performed by GC×GC/TOFMS allowed the tentative identification of 995 compounds. The main classes found in crude bio-oil were nitrogenous compounds, with emphasis on pyrroles (13.19%), nitriles (9.04%), pyridine (5.77%) and oxygenated compounds: phenols (10.27%) and carboxylic acids (4.48%). The increase in the separation power enabled the identification and separation into classes by groups of regions of the chromatogram, generating well-ordered 2D maps, which can be used to monitor the transformation process to which the oil may eventually be subjected.

The residual tobacco seeds (after the oil extraction) were pyrolyzed by Onorevoli et al. [46]. An acid-base extraction was performed on the bio-oil in order to obtain extract rich in nitrogenous compounds. The acidic organic phase was extracted using HCl and the nitrogen compounds were recovered using NaOH due to their basic characteristics. The crude bio-oil and the nitrogen-rich extract were subjected to analysis by GC×GC/qMS (qualitatively and semi-quantitatively). In the crude bio-oil, 148 compounds were tentatively identified, among them, phenols, esters, ketones, alcohols, hydrocarbons and nitrogen compounds; while in the nitrogen rich extract, 40 compounds (mostly nitrogen compounds) were identified.

Lazzari et al. [47] submitted mango seed residues (tegument and almond) to pyrolysis. The bio-oils obtained were analyzed by GC×GC/TOFMS, and 108 compounds were identified for the tegument bio-oil sample, with phenols (32.6%) and ketones (22.9%) as major classes. As for the almond bio-oil, 120 compounds were identified, with the major classes being ketones (20.6%) and acids (16.8%).

In another research developed by Silva et al. [48], the liquid products (bio-oil and aqueous phase) obtained through the slow pyrolysis of the crambe seed were analyzed. The organic compounds present in the aqueous phase were lyophilized and diluted in organic solvent for analysis. The elucidation of the chemical composition of the fractions was performed by GC×GC/TOFMS. For the bio-oil sample, an inverse set of columns (DB-17 as the first dimension column and a DB-5 as the second dimension column) was used, which is more suitable for hydrocarbon separation. In this case, using an unconventional set of columns, the separation in the first dimension is done through the volatility and specific interactions of the compounds, while in the second dimension the separation depends only on volatility. The bio-oil analyzed has more apolar characteristics, rich in hydrocarbons, which explains the use of an apolar column in the first dimension for better separation of this class. For the aqueous phase, the conventional system (DB-5 as the first-dimension column and a BPX-50 as the second-dimension column) was adopted, which is more suitable for the separation of organic compounds present in this phase due to the high polarity of their compounds. The identification of the compounds was done by comparing the mass spectra with the NISTTM library associated with the retention index. The semi-quantitative analysis was performed using the relationship between peak areas and the concentration of internal standards. The use of the unconventional set of columns allowed a better separation of the hydrocarbons present in large quantities (67.5%) from the total of identified analytes. Quantitative analysis allowed determining 66% in bio-oil mass. Among the identified classes, the alkyl-benzenes, nitriles and olefins displayed higher concentrations. In total, 137 alkyl-benzenes were identified, with toluene being the major compound (26.4 mg g^{-1}). In the aqueous phase, 136 compounds were identified, with the predominant classes being amides and carboxylic acids, with acetic acid being the major compound (48.7 mg g^{-1}). Semi-quantification by GC×GC/

TOFMS identified only 34.7% by mass, likely due to the highly polar characteristic of this type of sample and, consequently, the strong interaction with the stationary phase used in the chromatographic separation. The characterization of such complex samples was feasible due to the high resolution of the applied technique, which also allowed the use of tools such as spectral deconvolution in the separation of co-elutions of some compounds found.

Onorevoli et al. [49] also used crambe seed to obtain bio-oil by fast pyrolysis. However, before the pyrolytic process, the biomass was extracted with 3 extractive processes to remove vegetable oil: mechanical pressing extraction (MPE), Soxhlet extraction (SE) and compressed propane extraction (CPE). The analysis of bio-oil compounds was performed using GC/qMS and GC×GC/TOFMS techniques, using a conventional column set. The analysis showed similar chromatographic profiles for the three samples and through this it was possible to tentatively identify 195 compounds in the bio-oil obtained after the pressing extraction (MPE), 307 compounds for the bio-oil obtained after the Soxhlet extraction (SE) and, finally, 361 compounds in the bio-oil obtained by the compressed propane extraction (CPE). For the MPE sample, the amounts of acids and phenols were high, showing low efficiency in the extraction of vegetable oil, while the hydrocarbons class appears in higher concentrations for the SE sample. The main differences found between the analysis techniques are related to the classes of alcohols, aldehydes and nitrogen compounds. Alcohols and aldehydes do not appear in the analysis (or appear with small peak areas) by GC×GC/TOFMS, while the amount of nitrogen-compounds is greater when compared to the GC/qMS technique. This is probably due to the co-elutions that occur in GC/qMS, which result in peaks that are not completely separated, preventing the correct identification of the compounds.

In the research developed by Nunes et al. [50], the residual seed-cakes of soursop and bocaiuva (after oil extraction) were used in the slow pyrolysis process. Chromatographic analyses of the obtained bio-oils were performed by GC×GC/TOFMS. Semi-quantification was performed by relating the area of the identified peaks to some internal standards. Thus, 414 compounds were identified in the bio-oil sample of the soursop seed cake, with carboxylic acids (30.7%) and amides (25.4%) as major classes. As for the bio-oil of bocaiuva seed cake, 222 compounds were tentatively identified, with hydrocarbons and phenolic derivatives as major classes, these presenting areas of 32.0% and 29.4%, respectively.

GC×GC applied to bio-oils from other biomasses

Brazil is the world's largest sugarcane producer and it represents a large portion of energy production for industries. For this reason, several studies using residues of this biomass have been standing out in the last decade. Moraes et al. [51] applied the GC×GC/TOFMS technique for identification and semi-quantification of main compounds in bio-oil, derived from intermediate pyrolysis of sugarcane straw, allowing the tentative identification of 123 compounds. Cunha et al. [52] used pressurized solvent fractionation by solvent elution of the bio-oil obtained from the fast pyrolysis of sugarcane straw and analyzed the fractions by GC×GC/qMS. Using LTPRI, 166 compounds were identified. In both studies mentioned above, phenols, ketones, aldehydes and aliphatic hydrocarbons were predominant. Maciel et al. [53] performed extractions of the aqueous phase of the sugarcane straw bio-oil with solvents of different polarities, using SPE (Solid Phase Extraction) and LLE (Liquid-Liquid Extraction) techniques, and the analysis of the aqueous extracts occurred by GC×GC/TOFMS, which allowed the semi-quantitative identification of phenols, aldehydes and ketones mostly. Phenol was the main compound, indicating the potential use of this material as a source of phenolic raw materials for industry.

Mendes et al. [37] carried out a catalytic pyrolysis of sugarcane bagasse and the bio-oil produced was analyzed by GC×GC/TOFMS. Mostly, phenols, ketones, carboxylic acids, aliphatic and aromatic hydrocarbons were identified. Barros et al. [54] characterized the bio-oil of some species of sugarcane (straw and bagasse): Saccharum sp., Saccharum Robustum, Miscanthus sp. and Erianthus sp. using the GC×GC/qMS. The bio-oils presented similar chemical composition and the following compounds were mainly identified: phenols, methoxylated phenols, such as syringol and 4-vinyl guaiacol; furans, such as 2.3-dihydro-benzofuranand and in smaller proportions aldehydes, ketones and ethers.

Tessarolo et al. [41] submitted the sugarcane bagasse to the same pyrolysis process (thermal and catalytic) of pinewood (woody biomass) using ZSM-5 and the bio-oil obtained was also characterized by GC×GC/TOFMS. The samples presented similar profiles with pinewood and the classes identified were lactones, cyclic ketones, acids, aldehydes, phenols and aromatic hydrocarbons (which significantly increased with the use of the ZSM-5 catalyst).

Some studies with coconut fiber biomass have been discussed in recent years, due to the environmental concern that this waste generates by their disposal in open landfills after the coconut-water consumption. Almeida et al. [55] performed fast pyrolysis with green coconut fiber and the characterization of the bio-oil occurred by GC×GC/TOFMS, which allowed the semi-quantification of 94 compounds, mostly oxygenated.

Schena et al. [56] carried out comparative studies between the conventional GC×GC/TOFMS and fast-GC×GC/TOFMS for bio-oils samples from the fast pyrolysis of green coconut fiber. The results showed that in addition to a reduction in analysis time (around 80%) without compromising the separation of compounds, fast-GC×GC/TOFMS allowed a better identification of compounds and narrower peaks, increasing the signal/noise ratio. A total of 327 compounds were tentatively identified by comparing the mass spectrum of the compounds with the NIST™ library. Phenols, ketones, aldehydes and fatty acids were the major chemical classes found in both samples, followed by alkanes and aromatic hydrocarbons, esters and nitrogen compounds. In another study done by Schena et al. [57], two forms of optimization were carried out in the coconut fiber pyrolysis process. One of them was the alkaline extraction of the coconut fiber bio-oil, producing an acidic and neutral fraction. The other optimization process was a pre-treatment of biomass before pyrolysis, using two different extraction techniques (Soxhlet and Sonication). Both processes proved to be efficient and complementary: in the first case, there was a pre-concentration of the phenols in a single fraction and in the second, a large part of the fatty acid derivatives were removed from the bio-oil. These results indicated that the two techniques can be used to improve the quality of the bio-oil produced, removing free fatty acids from the biomass, and isolating compounds with high added value (in the case of phenols). In a third study by Schena et al. [58], the authors evaluated the effect of the TOFMS data acquisition rate on the quality of the analytical information obtained by GC×GC/TOFMS. In the analysis of coconut fiber bio-oil under fast GC×GC/TOFMS conditions, use of high data acquisition rates (200–300 Hz) increased the number of identifiable peaks by more than 50% compared with that achieved at the conventional rate of 100 Hz. The acquisition rate can affect the peak capacity by a factor of 3 or more. For quantitative analyses, it is possible to work with lower acquisition rates, as the number of data points per peak (DPPP) is maintained. This is because the peaks have high intensities, which is an important parameter in this type of analysis. In the case of qualitative analysis, it is important to consider two parameters that directly influence the quantity of identified compounds. The first is S/N ratio. Despite higher signal intensities, lower acquisition rates result in inappropriate S/N ratios for qualitative analysis, mainly owing to the higher noise signal. The same tendency is observed for co-eluted peaks. Higher co-elution degrees were found at 30 Hz, indicating that higher acquisition rates were required. Consequently, in bio-oil qualitative analyses, it is important to work with acquisition rates greater than 200 Hz. This was the first study to demonstrate the importance of optimizing the data acquisition rate, a parameter that has previously been neglected in the literature, in GC×GC/TOFMS development.

Moraes et. al. [59] characterized the bio-oil from the fast pyrolysis of pulp from the industrial processing of oranges by GC×GC/FID e GC×GC/TOFMS. The authors cited the separation in the second dimension and the use of the spectral deconvolution software (to TOFMS) as advantages to characterize the bio-oil and identify a greater number of compounds with co-eluted peaks separation. The GC×GC/FID system was used to optimize the chromatographic process and identify compounds using TOFMS detector. The following parameters were evaluated: temperature setting of the primary furnace, temperature difference between the primary and secondary furnaces (ΔT), modulation period (MP), modulator temperature and duration of the hot jet. With the best conditions defined, 167 compounds were tentatively identified, 26 of which were found with concentrations greater than 1% by GC×GC/TOFMS. The main classes were: acids, aldehydes, alcohols, ketones, phenols, ethers and nitrogen compounds.

Faccini et al. [34] carried out qualitative and semi-quantitative characterization by GC×GC/TOFMS of the bio-oils from two residues originated in the cellulose industry: digester residue (DR) and wastewater treatment sludge (WTS). The total number of compounds tentatively identified in the DR and WTS were 257 and 536, respectively. Phenols and ketones were the major chemical classes in the bio-oil of the DR, while the WTS showed a more complex chemical composition, including a greater variety of chemical classes. In addition to phenols, ketones, nitrogen compounds, alcohols, aliphatic and cyclic hydrocarbons, it was possible to identify the formation of polycyclic aromatic hydrocarbons, which represented 7.6% of the total of tentatively identified compounds. The highest percentage of PAH area corresponded to naphthalenes (4.3%), followed by indenes (2.1%) and indanes (0.5%).

Lazzari et al. [60] studied the intermediate pyrolysis of rice husk and characterized (qualitatively and quantitatively) the organic phase of the bio-oil by GC×GC/qMS. Through the analysis, a total number of 98 compounds was found and 62 were quantified using a developed quantitative method using relative response factors (RRFs). Phenols and ketones (cyclics) were predominant in the organic phase, with 8.21 and 5.9 wt%, respectively; and benzofuran (1.37 wt%) corresponded to the main compounds identified. Rice husk was also the subject of the study by Moraes et al. [45]. For the pyrolysis process, 2^3 factorial planning including the following parameters: granulometry, nitrogen flow and mass of rice husk. The best conditions were applied to the production of bio-oil, which was characterized qualitatively and semi-quantitatively using GC×GC/TOFMS. In total, 106 compounds were identified from the classes of phenols and ketones (major classes), acids, ethers, aldehydes and alcohols. The major compounds found in this sample were guaiacol, contributing to 14.14% of the total area of the identified peaks. In another study by Lazzari et al. [61], for the first time in literature, a systematic approach employing matrix-matched calibration was presented to evaluate the extent of matrix effect in bio-oil analysis and undoubted quantification of its components. A procedure using sequential liquid-liquid extraction (LLE) based on two approaches (organic solvent partitioning and pH-depended reactive extraction) was performed to obtain a blank bio-oil matrix. In order to assess matrix effect, two types of external calibration were employed, namely, matrix-matched calibration and solvent calibration. The procedures proved to be efficient in the extraction of target chemical classes by bio-oil and the evaluation made from the two external calibration methods, allowed to observe the dependence of the matrix effect in the analysis of bio-oil by gas chromatography. This method of matrix-matched calibration was implemented in the quantification of bio-oil by GC×GC/qMS. Were identified 82 compounds in the rice husk bio-oil, 52 of which were quantified using the method of matrix-matched calibration. Ketones (110.1 g kg^{-1}) and phenols (109.2 g kg^{-1}) were majorly present in the bio-oil, together these two classes correspond to 79% of the quantified of the sample. The authors noted that some very important compounds quantified in the rice husk bio-oil showed matrix effect.

The residues of bocaiuva (endocarp, bark and fiber) were submitted to pyrolysis at different temperatures by Cardoso et al. [62]. The bio-oils were subjected to chromatographic analysis using the GC×GC/TOFMS technique. In total 151, 111 and 78 compounds were tentatively identified for the bio-oil of the endocarp (phenol being the major compound with 19.64%), bark (guaiacol being the major compound with 21.98%) and fiber (toluene being the major compound with 50.96%) of bocaiuva, respectively. Some compounds were quantified with commercial standards and their values expressed in g of the compound per g of bio-oil. For the bio-oil of endocarp, bark and fiber of bocaiuva, phenol (0.13 g g^{-1}), furfural (0.11 g g^{-1}) and toluene (0.28 g g^{-1}) were identified in greater concentration for each bio-oil, respectively. Despite the superior performance of the chromatographic technique used, some compounds showed co-elution in both dimensions. In these cases, the use of the spectral deconvolution tool was employed promoting the separation of some compounds by differentiating the mass spectra and retention times.

POSSIBLE APPLICATIONS OF BIO-OILS

The bio-oils analyzed show differences in their composition and concentration of chemical compounds classes. This diversity of chemical compounds makes it possible to suggest its application in the most diverse branches of the fine chemical industry or even in the energy sector. Hydrocarbons, for example,

can be applied as an alternative to fossil fuels, after an adequate up-grade. Oxygenated compounds, such as phenols, are important inputs in the chemical industry for the production of polymeric resins, pesticides, dyes and explosives, as well as nitrogen compounds are widely used for syntheses in the pharmaceutical industry. Compounds derived from aldehydes, such as furfural, can be purified by going through hydrogenation processes and generating high added value products for application in lubricants, plastics, nylon and adhesives. Fatty acids and esters, identified in some samples (mainly oily), can be used in the production of biodiesel. In Figure 5, the main chemical classes found in bio-oil can be observed in all studies with Brazilian biomasses with potential industrial use [63-66].

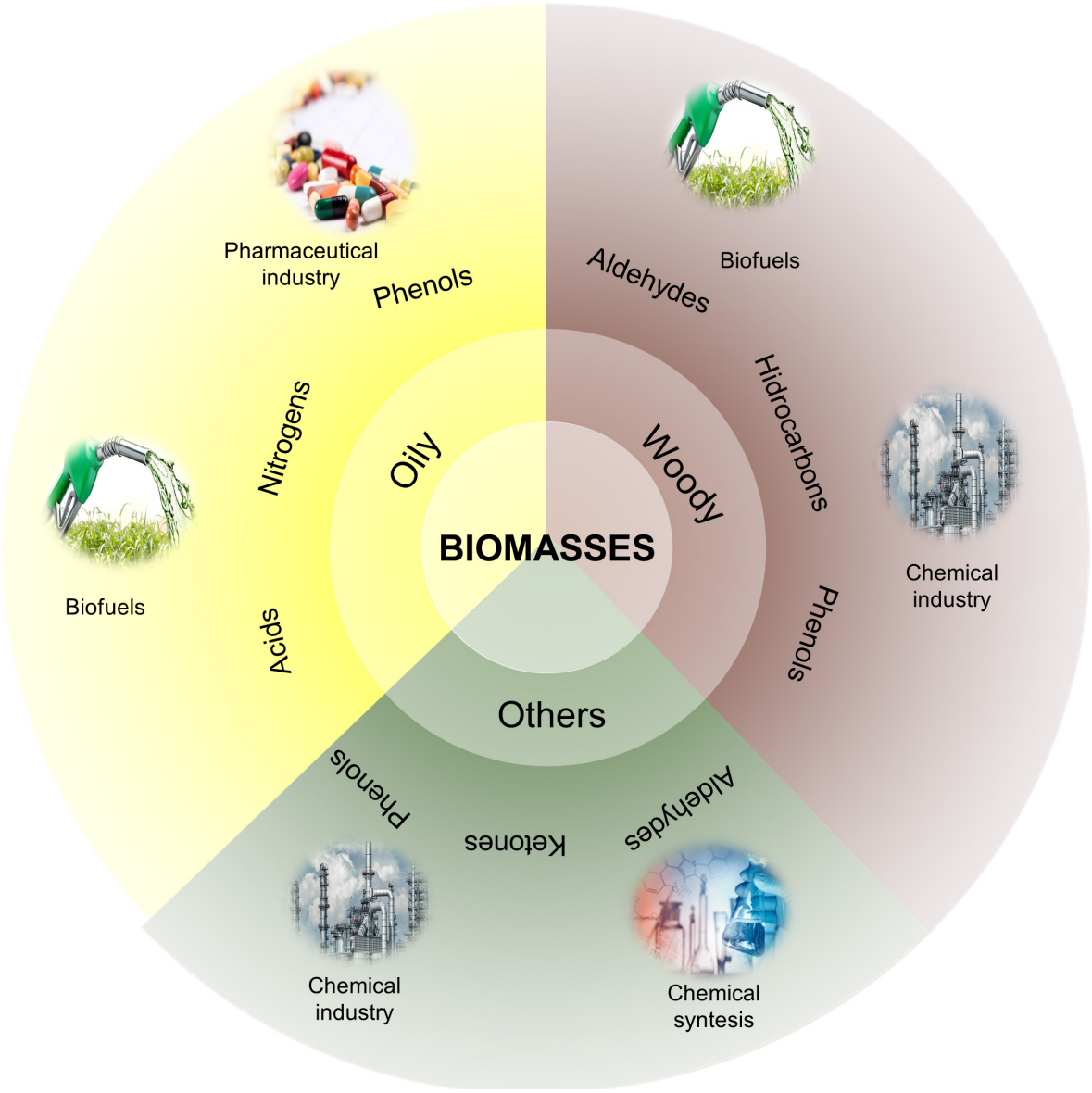

Figure 5. Main classes of compounds found in bio-oils according to the type of biomass and its possible applications.

CONCLUSIONS

This study reveals the state of the art of chromatography developed in Brazil. GC×GC emerges as an extremely important tool in the separation and proper identification of substances, greatly reducing the misidentification with false positives that the one-dimensional technique still allows.

As can be seen, most of the work is related to the application of this technique in the analysis of typical samples of the Brazilian agroindustry, which can assist in the research and use of these important residues that cause environmental impact. The most complete identification, provided by the best separation and by very sensitive and high-resolution detectors, allows the indication of use and consequent application of this bio-oil in the respective industries (food, drugs, chemicals, polymers, ...) to be more effective.

Another important evidence is the high qualification of Brazilian science in chromatographic separation techniques, with laboratories equivalent to the main in the world in the area of GC×GC, including several universities and research centers.

Related to the evolution of GC×GC and its application to biomass derivatives, two paths for the next steps can be proposed: quantitative analysis and rapid analysis. Quantitative analysis requires the use of standards, which is unconventional when talking about more than 200 identified compounds. Furthermore, the type of integration is fundamental, it is possible to treat the “compounds” as peaks, bands or pixels. In this sense, researches have been developed with the objective of recognizing families of compounds and using an approximate response factor for each family. This approach can minimize the use of patterns and optimize analysis time. It is necessary to use peak-to-peak integration, as shown in works throughout the discussion. This procedure still needs further studies to allow its automation, through available software. In fact, the availability of good software is one of the points to be improved in GC×GC, whether in terms of efficiency or acquisition cost.

On the other hand, just as single-dimensional chromatography evolved into fast columns, GC×GC also demonstrated the possibility of using these columns in their first dimension. This makes the characterization of bio-oils by GC×GC more efficient and competitive.

Conflicts of interest

This research has no conflict of interest and no special funding as destined to it. This article does not contain any studies with human participants or animals performed by any of the authors.

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