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SUGARCANE SPIRITS (CACHAÇA) QUALITY ASSURANCE AND TRACEABILITY: AN ANALYTICAL PERSPECTIVE

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11.1 Introduction

According to the Brazilian legislation, sugarcane spirit (cachaça) is a typical beverage exclusively produced in Brazil with alcohol content between 38% and 48% in volume, at 20°C. It is obtained from the sugarcane fermented wort, showing peculiar sensory characteristics. It is allowed to include up to 6 g L^{-1} of sugars, expressed as sucrose (Brasil Ministry of Agriculture—MAPA, 2005).

According to the Brazilian Ministry of Agriculture, Livestock, and Supply, the congeners coefficient or secondary composition of cachaça cannot be lower than 200 mg or higher than 650 mg for each 100 mL of ethanol. As conformity control, the legislation establishes limits for some chemical compounds (Table 11.1). Therefore, the control of potentially mutagenic and carcinogenic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and pesticides residues, as for example, should be essential to guarantee the quality of the cachaça and consequently the consumer's health (Galinaro et al., 2015; Serafim and Lanças, 2018).

Cachaça is produced in all Brazilian states. The main producers of cachaças are: São Paulo-SP (45%), Pernambuco-PE (12%), Ceará-CE (11%), Rio de Janeiro-RJ (8%), Minas Gerais-MG (8%), Goiás-GO (8%), Paraná-PR (4%), Paraíba-PB (2%), and Bahia-BA (2%). Brazil has a cachaça production capacity of approximately 1.2 billion L per year, which is represented by more than 4000 different brands and

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Table 11.1 Congeners and Contaminants Monitored in Cachaça According to the Brazilian Legislation

	Concentration
Congeners	
Volatile acidity (as acetic acid)	150 mg[a]
Esters (as ethyl acetate)	200 mg[a]
Aldehydes (as acetaldehyde)	30 mg[a]
Furfural and 5-hydroxymethylfurfural	5 mg[a]
Higher alcohols	360 mg[a]
Contaminants	
Urethane	150 μg L^{-1}
Acrolein	5 mg[a]
Methyl alcohol	20 mg[a]
2 Butyl alcohol	10 mg[a]
Cooper	5 mg L^{-1}
Lead	200 μg L^{-1}
Arsenic	100/L

[a]In 100 mL of anhydrous alcohol (AA).

approximately 15,000 cachaça production plants in the country. According to the *Instituto Brazileiro de Cachaça* (Brazilian Institute of Cachaça), in 2016, a large volume of cachaça was exported to 54 countries generating revenue of more than US$13.8 million. Germany, EUA, Paraguay, Uruguay, and France were the main importers countries (Fig. 11.1).

11.2 Chemical Composition of Sugarcane Spirits

In sugarcane spirits and other distillates in general, the presence of high amounts of volatile chemical compounds can contribute with the product quality. Esters, for instance, are considered as important aroma components due to their low odor and flowery threshold. Its production is associated with the yeast lipid metabolism (alcoholysis of acyl CoA compounds) or, in lower extension, by esterification reaction between fatty acids and alcohols.

Among the most abundant esters in alcoholic beverages and their respective sensorial proprieties are ethyl acetate (fruity), ethyl lactate (mild, buttery, creamy, with hints of fruit, and coconut), isoamyl ac-

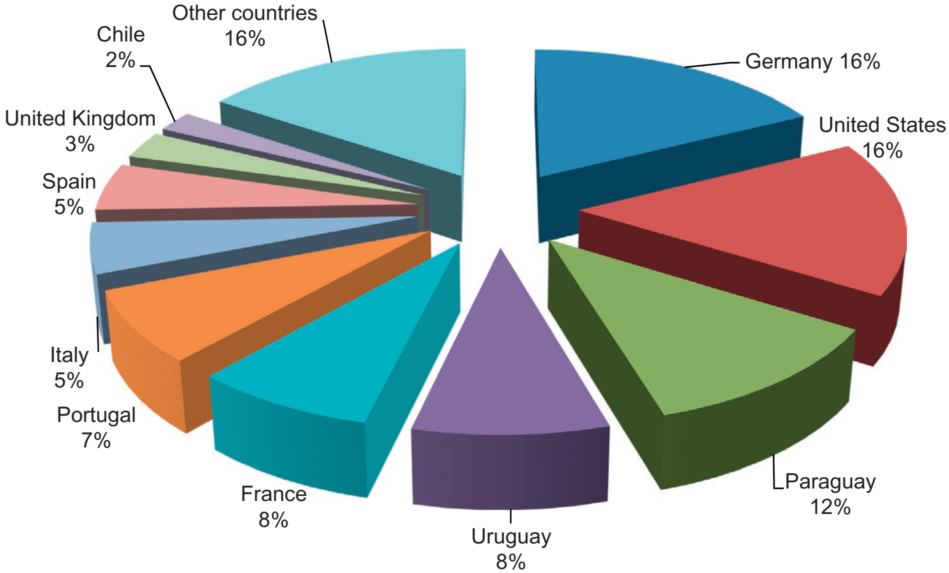

Fig. 11.1 Main countries importers of cachaças from Brazil.

etate (banana), benzyl acetate (apple), ethyl hexanoate (apple and aniseed), ethyl octanoate (fruity and fat), and ethyl decanoate (brandy-like), isoamyl octanoate (apricot, melon, and mango), and ethyl dodecanoate (apple, apricot, guava, and melon).

The major volatile acidic compound responsible by the distillate's acidity is acetic acid, followed by lactic acid. But other carboxylic acids such as formic, propionic, butyric, succinic acid, citramalic, and longer chain fatty acids such as capric acid, lauric acid, myristic acid, and palmitic acid also are involved in the cachaças (Serafim et al., 2012). The volatile acids have specific odors but its influence happens only when present at high levels, indicating a microbial spoilage (Serafim et al., 2012). Gallic acid, vanillic acid, and syringic acid are considered aging markers (Alcarde et al., 2014).

Some alcohols are produced mainly during fermentation (e.g., ethanol) and can contribute negatively with the strong pungent smells at higher concentrations but contribute more positively when present at lower concentrations. However, hexanol, benzyl alcohol, and 2-phenylethanol are from the raw material. Bagasse residues are the major responsible for high concentration of methanol in the sugarcane spirits due to the presence of pectin. The most abundant alcohols in cachaça are isoamyl alcohol, 1-propanol, 1-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol.

Aldehydes and ketones can contribute to unpleasant green notes in wine, whisky, and Cognac (Ledauphin et al., 2004; Hashizume and Samuta, 1997; Wanikawa et al., 2002; Johnson et al., 2017; Awad et al., 2017). Aldehydes found in alcoholic beverages such as formaldehyde, acetaldehyde, acrolein, butiral and isobutiraldehyde, valeraldehyde, and benzaldehyde diacetyl are produced during fermentation or by processing (furfural and 5-HMF—the Maillard reaction), or during the aging process during the wood cask extraction such as cinnamaldehyde, syringaldehyde, coniferaldehyde, synapaldehyde, and vanillin (Alcarde et al., 2014). Acetaldehyde, the main aldehyde in the distillate, is usually considered an off-odor with unpleasant and pungent odor as sensorial characteristics. For this, the balance between the ethyl acetals and acetaldehyde is very important since acetals have pleasant and fruit aroma (Alcarde et al., 2014).

The origin of ketones can be associated with secondary fermentation processes and possible contamination during production or derived from the raw material as, for example, the damascenone (exotic flower or rose). The production of diacetyl (2,3-butanedione) can be explained by the lipids auto-oxidation, particularly of unsaturated fatty acids. The ketones usually do not present sensory significance attributes, but diacetyl has been shown to be a key odorant compound in freshly distilled Cognac spirit (Ferrari et al., 2004).

Sulfurous compounds are usually derived from yeast metabolism and from amino acids nonenzymatic reactions (such as cysteine and methionine), the peptide glutathione, the vitamins thiamine and biotin, thiols, and a wide diversity of volatile compounds present during the fermentation process. The major volatile sulfur compounds present in cachaças are dimethyl sulfide (DMS), which exhibits a strong negative influence on the beverage sensory qualities. Although they are found in trace amounts, their low sensory thresholds can give them great significance. Its odor threshold of $25\,\mu g\,L^{-1}$ has been determined in several wine samples. It is more present in the nonaged cachaças than in the aged ones, which could be partially explained by the high DMS volatility (b.p. = 38°C) leading to its concentration decrease during the aging process.

Terpenes are a large group of natural compounds being common constituents of flavorings and fragrances and consequently showing important properties in the food science, cosmetics, pharmaceutical, and biotechnology areas. Terpenes are generally found in essential oils obtained from flowers and fruits and their concentration has been used to identify different grape varieties and to attest the sensorial quality of wines, beers, and spirits and distils. They are originated from yeasts during the fermentation process and their concentrations are limited to trace by a small number of *Saccharomyces cerevisiae* and non-*Saccharomyces* species such as *Kluyveromyces lactis*, *Torulaspora delbrueckii*, *Kloeckera apiculata*, *Metschnikowia pulcherrima* and *Candida stellata*, and *Ambrosiozyma monospora* (Drawert and Barton, 1978; Gounaris, 2010; Sadoudi et al., 2012; Wu et al., 2015).

11.2.1 Toxic Contaminants

Toxic substances reported in the literature found in the alcoholic beverages include: metals (e.g., Pb and As), chloropropanols (CPs), acrylamide, furan-derived compounds, phthalate esters (PAEs), PAHs, mycotoxins, pesticides, and ethyl carbamate (EC) (NJayakody et al., 2016; Mottram et al., 2002; Pflaum et al., 2016; Bekatorou, 2016).

The soil composition, environmental conditions, agrochemical treatments all can affect the mineral content in the raw materials (sugarcane in this case). The presence of metals into alcoholic beverages is associated with all stages of production, including the equipment used during the distillation process (cooper alembic or steel column), bottling, and aging process.

CPs, PAEs, and acrylamide are compounds considerate as potential carcinogens for human's health and their contamination are one of the hot topics related to food safety. CPs are formed from the acid-hydrolyzed vegetable protein process used as ingredient in the non-fermented soy sauce production (Baer et al., 2010; Moa et al., 2014). Acrylamide, like furans compounds, is a by-product of the Maillard reaction and may be formed during the food toast or frying process (Mottram et al., 2002; Pflaum et al., 2016; Bekatorou, 2016; Moa et al., 2014). CPs and acryl amide, until now were not identified in sugarcane spirit samples.

Contamination by phthalate compounds in food and beverages are normally attributed to their association with plastics such as polyvinyl chloride, polyethylene, polyethylene terephthalate, and polyvinyl acetates. The high ethanol concentration may extract lipophilic PAEs during the contact with plastic materials (De Souza et al., 2009a, b; Bekatorou, 2016). Phthalate has been qualitatively identified in sugarcane spirits samples using comprehensive two-dimensional (2D) gas chromatography (GC) (De Souza et al., 2009a, b).

Mycotoxins such as ochratoxin A and aflatoxins B1, B2, G1, and G2 are frequently associated with the raw material contamination used in the distilling industry (Kłosowski and Mikulski, 2010). In sugarcane grass and juice were detected the presence of aflatoxin B_1 and aflatoxin G_1 (Abdallah et al., 2016). However, there are no reports on the presence of mycotoxins in sugarcane spirits. For other distillates, such as Barley Shochu, it was demonstrated that the distillation step effectively reduces the contamination risk (Nagatomi et al., 2012; Bekatorou, 2016).

Pesticides are considered harmful products that can cause adverse effects on the human health. Recent studies suggest that environmental contaminants, including pesticides, might play an important role in the pathogenesis of diabetes (Firmin et al., 2016; Kuo, 2013; Magliano et al., 2014). The extensive use of pesticides by farmers in agriculture aiming to protect crops against pests, diseases, and weeds in modern agricultural practices to ensure greater efficiency in food production has been a constant concern by consumers. The usual and incorrect application of pesticides in the agriculture can cause contamination of soil, atmosphere, foods, surface, and groundwater. Consequently, consumers' intoxication can occur through consumption of contaminated foods (Yongtao Han et al., 2016).

Recently, the presence of tebuthiurom, carbofuran, simazine, atrazine, metribuzin, ametrin, hexazinone, bifenthrin, and diuron residues was evaluated in sugarcane spirits samples using a simple analytical method based in GC coupled to triple quadrupole mass spectrometry (GC-MS/MS). In this case, hexazinone ($20.0\,\mu g L^{-1}$), bifenthrin ($18.8\,\mu g L^{-1}$), and diuron ($14.3\,\mu g L^{-1}$) were detected in nonaged commercial samples. Although just traces of pesticide residues have been found in the investigated samples, the results point out that the consumers of cachaça should be concerned about the food safety aspects of the product consumed since pesticides may pass by the distillates when present at very higher concentrations during the treatment of sugarcane plantation (Serafim and Lanças, 2018).

EC, also known as urethane, is generally found in fermented foods (bread, yogurt, wine, and beer) and in distilled spirits (whisky, cachaças, rum, vodka, grappa, and tiquira) and may be correlated to a carcinogenic effect. Canada, the United States, France, Germany, and Switzerland established specific laws in order to control the presence of EC in alcoholic beverages. In Brazil, the suggested limit for EC control in sugarcane spirits is $150\,\mu g L^{-1}$.

The fermentation and distillation steps are responsible for the presence of EC in the spirits. The distillation process should reduce the presence of EC in the distillates since EC present a relatively high boiling point (≈182°C). Thus, a still unknown reaction pathway should explain the EC presence in the distillated. Molecular modeling [density functional theory (DFT)] analyses showed that during the EC formation, isocyanic acid (HNCO) is an active EC precursor synthesized after the distillation process and in the ethanol presence, resulting in the EC formation (Galinaro et al., 2015). The reaction rate is slow and strongly influenced by the pH, ethanol concentration, and temperature of the solution. Galinaro and Franco (2011) evaluated the stability rate of EC formation. The concentration of EC reached a maximum value after 15 days of distillation. Thus, after reached the EC stability, a simple redistillation, showed a reduction of its concentration to 92.5% (Galinaro and Franco, 2011).

11.3 Differentiation Between Cuban Rum and Brazilian Cachaça

Due to the rapid increasing on cachaça internationalization, already the third distillate most consumed in the world, the Brazilian product was at the beginning confused with the rum traditionally produced in Caribbean countries, being considered a lower quality product. Cachaça is the typical Brazilian distillate denomination of the spirit produced from the distillation of fermented sugarcane juice, whereas rum is a sugarcane spirit obtained by the distillation of molasses, a cooked byproduct of sugar production, and distilled to obtain much higher percentages of alcohol by volume. Thus, the must composition, as well as variations in the production process could influence their chemical profiles. Cardoso et al. (2004) distinguished 18 cachaças from 21 rum samples using chromatographic methods [GC-MS, GC-flame ionization detector (FID), and high-performance liquid chromatography (HPLC)-UV-vis] and inductively coupled plasma atomic emission spectrometry in order to compare their chemical composition (octanoic acid, nonanoic acid, decanoic acid, and dodecanoic acid, gallic acid, protocatechuic acid, epicatechin, vanillic acid, syringic acid, syringaldehyde, vanillin, pcoumaric acid, coniferyl aldehyde, sinapaldehyde, ellagic acid, coniferyl alchool, kaempferol, and quercetin, Al, Ca, Co, Cu, Cr, Fe, Mg, Mn, Ni, Na, and Zn). According to their results, the method described showed to be useful in cachaça and rum discrimination (Cardoso et al., 2004). A fingerprint approach was used to distinguish these distillates by direct-infusion employing electrospray MS in the negative ion mode, DI-ESI (−) MS. It was possible identify each type of sample according to their processing. For example, cachaças aged in *amburana* and *jequitiba* casks presented characteristics spectral mass (m/z 271, 313, 377), (m/z 171, 255, 455), respectively, while the mass spectra of commercial rum samples showed as characteristic ions having m/z 89, 97, 179, 255, and 283 (De Souza et al., 2007). Consequently, these results aided to obtain from the international community the recognition and acceptance that cachaça is a product of Brazilian exclusivity. Until 2013, in the United States markets, the cachaça bottles were labeled as "Brazilian rum," but in 2014, an agreement between Brazilian and EUA governments established that all Brazilian sugarcane spirit must be named cachaça.

11.4 Chemical Traceability of Cachaça's Steps of Production

Traceability is a relevant certification information at every step of the production of foods, beverages, and other products. Ensuring the reliability of certified labeling highlights the quality of the products, its traceability, and consequently, consumer protection. Chemical information on the composition of Brazilian sugarcane spirit can provide important data about it production steps. As there is no standard method available to produce cachaças, different processes can be used thus resulting in beverages with varying chemical compositions.

11.4.1 Harvest of Sugarcane Crops

The main steps of cachaça production are: the sugarcane (*Saccharum* spp.) harvest, the sugarcane juice extraction, must preparation, fermentation, distillation, and aging.

Different sugarcane hybrid species can be used to produce cachaça; generally, stout produces an abundant sweet juice, while culms are thick and soft due to low fiber content. Sugarcane is not demanding of any specific climate conditions, high soil fertility, and water supply, besides presenting good resistant to diseases such as mosaic, gummosis, leaf scorch, and root rot.

The influence of five different sugarcane cultivars and degree of maturation (three harvest seasons) on the chemical profile of cachaças produced under strict controlled conditions was recently evaluated. Chemometric tool did no show correlation between the chemical composition and the type of cultivar at the same harvest stage. The production of acetic acid, ethyl lactate, and n-butyl alcohol was higher in the first harvest (June) when compared to the others (Fernandes et al., 2017). However, this chemical compounds are traditionally correlated with bacterial contamination during the fermentation process.

Sugarcane can be harvested either manually or mechanically. Hereafter, the manual harvesting accounted for more than half of the Brazilian production but nowadays this method is restricted to areas with hard access and high slope. In order to facilitate the manual harvest and to protect rural workers from sharp leaves, insects, and poisonous snakes, while increasing the sugar concentration by evaporating the water in the stalk, sugarcane plantation is burnt. Therefore, carcinogenic and mutagenic PAHs, formed during incomplete combustion or pyrolysis of organic matter, are adhered to the sugarcane stalks resulting in contamination of the juice during processing; consequently these compounds could be present in the distillated. The increase risks of bladder, skin, and lung, cancers when there are high occupational exposures to PAHs have been confirmed by epidemiological studies (Luch and Baird, 2010). PAHs have been found as contaminants in different food categories such as smoked meat and fish (Zachara et al., 2017), canola, sunflower and corn oils (Molle et al., 2017), milk, meat, fish (Santonicola et al., 2017), and yogurt (Battisti et al., 2015). Tfouni et al. (2007) developed a methodology based on liquid chromatography with fluorescence detection to identify the presence of five PAHs (benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, and dibenz[a,h]anthracene) in 25 brands of cachaça commercially available in Brazil. Galinaro et al. (2015) evaluated the possibility to correlate the concentration of naphthalene (NA), acenaphthene (AC), fluorene (F), phenanthrene (PA), anthracene (A), fluoranthene (FL), pyrene (P), BaA, chrysene (CH), BbF, BkF, BaP, DBahA, benz[g,h,i]perylene (BghiP), and indeno[1,2,3-c,d]pyrene (IP) with cachaças produced from sugarcane harvested from fields set on fire or not.

Thus, by the PAHs quantitative analytical data combined with chemometric tools allows the identification of cachaça produced from sugarcane burned crops, thus making this analytical protocol useful as a routine method for traceability purpose (Galinaro et al., 2015).

11.4.2 Fermentative Process

Alcohol fermentation or ethanol fermentation is the anaerobic (nonoxygen requiring) pathway carried out by yeasts where sugars (generally glucose) are converted in ethanol and byproducts. The process of alcohol fermentation can be divided into two steps. In the first part, known as glycolysis, the yeast breaks down one mol of glucose to form two moles of pyruvate. These are converted into 2 mol of carbon dioxide and 2 mol of ethanol (fermentation). This process can occur even under adverse conditions, due to the high yeasts adaptive ability. However, this robustness may lead to the neglect of the fermentation process causing prejudice to sensory quality of distillate.

Traditionally during the fermentative process are used two types of ferments: the "*natural*" or "*endogenous*" and the "industrial" yeast, both belonging to the *S. cerevisiae* strains. The "*natural*" or "*spontaneous*" fermentation involves the natural inoculation of sugarcane juice by microorganisms present in the local environment. Since the amount of *S. cerevisiae* strains in this environment by itself is not enough, a method known as "*pé-de-cuba*" is required to increase their population. In this procedure, rice bran, corn flour, or soybean, are mixed with the sugarcane juice, as nutritional supplements to support the reproduction of the natural *S. cerevisiae*. During the spontaneous fermentation process, *non-Saccharomyces strains* and others microorganism like lactic and acetic acid bacteria and fungus, can also naturally inoculate the wort and contribute with the chemical complexity of the distillate. Schawn et al. (2001) found a great variety of yeasts strains such as *S. cerevisiae*, *Kluyveromyces marxianus, Pichia heimii, Pichia subpelliculosa, ebaryomyces hansenii,* and *Hanseniaspora uvarum* microorganism in the must during the alcohol fermentation processes. Gomes et al. (2010) isolated lactic acid bacteria along the whole fermentative processes when utilizing alembic cachaça production. *Lactobacillus plantarum* and *Lactobacillus casei*, *Lactobacillus ferintoshensis*, *Lactobacillus fermentum*, *Lactobacillus* *jensenii*, *Lactobacillus murinus*, *Lactococcus lactis*, *Enterococcus* sp., and *Weissella confusa* were the species isolated. The by-products from fermentation depend mainly on microbiota present in the must. However, factors as raw materials, pH, and temperature control should also be considered. Among the by-products of alcoholic fermentation, known as congeners, the fatty and volatile organic acids, esters, aldehydes, ketones, terpenes, alcohols, sulfur compounds, and glycerol are the compounds class more representative in the distillate (Garcia et al., 2015; Serafim et al., 2012, 2013, 2016, Serafim and Franco, 2015). It has been observed in whiskies and cachaças production that depending on the level of contamination, occur the formation of "*off-flavors*" compounds and changes in the distillate yield (Wilson, 2014; Carvalho et al., 2015; Duarte et al., 2011, 2013). The natural fermentation is more commonly used in traditional (artisanal) production, but they can also be used in large scale as well (Lima, 1999; Pataro et al., 2000; Maia and Campelo, 2006).

Either isolated yeasts strains or genetically modified ones are more commonly used in industrial scale in the Brazilian distilleries neglecting the diversity of native yeast strains that are responsible for the production of cachaças peculiar flavors. In this case, the required amount of isolated yeasts strains necessary to start the alcoholic fermentation step is used, thus avoiding yeasts multiplication and the consequent contamination risks. There is currently a growing concern with the isolation of industrial yeasts strains in order to improve their capacities to produce chemical compounds that positively contribute to the sensory qualities of distillate and increases the process yields.

The traceability of indigenous and commercial *S. cerevisiae* strains has also been done in the winemaking (Alves et al., 2015; Tofalo et al., 2016). A large number of commercial *S. cerevisiae* strains have been used around the world for wine production and its metabolome was correlated to assess interstrains variability. For this purpose, 257 volatile metabolites belonging to classes of acetals, acids, alcohols, aldehydes, ketones, terpenic compounds, esters, ethers, furan-type compounds, hydrocarbons, pyrans, pyrazines, and sulfurous compounds were identified and related with metabolic pathways of amino acid, carbohydrate and fatty acid metabolism as well as with the mono and sesquiterpenic biosynthesis (Alves et al., 2015; Tofalo et al., 2016). However, in the wine production, differently from the cachaça process, there is no distillation step where many chemical compounds could be lost thus prejudicing the fermentation traceability.

Identification of the yeast strains in the cachaça production has been carried out through yield, conversion, efficiency, and microbiological analyses of the fermentative process. However, these studies did not take into consideration the chemical modification in the cachaça properties. It was recently described a correlation between the chemical profile of cachaças and the use of different commercial and autochthonous *S. cerevisiae* yeast strains. The cachaças obtained from fermentations with CA-11, BG-1, and CAT-1 yeasts presented chemical composition according to the limits established by the Brazilian legislation. The BG-1 strain had the highest volatile acidity, which is a parameter associated with a sensorial defect in distilled beverages. The CA-11 strain produced the distillate with the best chemical composition related to sensorial quality. Regarding to the autochthonous yeast species, the role of the native microbiota showed itself a strong element to be considered in the chemical profiling of the distillate and traceability of cachaça's territory, or terroir.

It was evaluated a traceability of the natural and "industrial" fermentative process of cachaças distilled in the steel columns through chemical analysis. In this case, the "industrial" ferment employed was the same as that utilized in the bread production. The cachaças produced with natural fermentation presented higher concentrations of total ester concentrations with the predominance of ethyl lactate and ethyl acetate. The contents of ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl octanoate, and ethyl decanoate were higher in the cachaças originated from industrial yeasts than in the other samples. The concentrations of acetic acid and DMS are about 10-fold higher in the cachaças from natural yeasts than in those produced with industrial yeasts. The differences between both fermentative processes are responsible by the chemical profile of distillates. The rice bran, corn flour, or soybean used as supplements to support the reproduction of the natural *S. cerevisiae* are source of amino acids that when metabolized generate target compounds characteristic of natural fermentation, such as DMS. The highest concentrations of acetic and lactic acids are probable due to bacterial contamination during the natural *S. cerevisiae* proliferation. These results contributed with the discrimination of *S. cerevisiae* strains used during the fermentation of cachaças distilled in columns. Regarding to the cachaças distilled in alembics, the concentration of DMS, acetic, and lactic acids did not allow the discrimination of fermentative step due to particularities of the distillation process. In the alembic distillation, there is the "*cutting process*" where the distillate is separated into three fractions ("tail", "heart," and "head"). However, only the "heart" fraction is commercialized. Each fraction presents a characteristic chemical profile was the DMS is more representative in the "head" fraction, and acetic and lactic acids in the "tail" fraction (Serafim et al., 2012; Serafim and Franco, 2015).

11.4.3 Distillation of Brazilian Sugarcane Spirits

By itself, nature cannot produce alcohol stronger than 14%. Thus, to obtain cachaças with alcohol content between 38% and 48% v/v, a distillation step must be included in the process. As in other distilled beverages, a distillation process in the production of cachaças is used to select and concentrate some specific volatile components of wine (sugarcane juice fermented) using heating. In general, the distillation process produces two types of distillates. Vodka and gin, as examples, are produced by rectification where repeated distilling and sometimes filtration are required, removing most of the flavor compounds. Cachaças, rum, whisky, and tequila are produced according to the specific distillation techniques that allows retention of the intrinsic raw material flavors (Bekatorou, 2016).

This heat can promote the Maillard reaction, a chemical reaction in which compounds such as acrolein, furfural, 5- hydroxymethylfurfural (5-HMF), pyrazines, and pyridines can be synthesized in the sucrose presence. Besides that, distillation causes the extraction of certain long-chain esters retained in the yeast cells at the end of the fermentation step, transferring them to the distillates (Léauté, 1990; Nykänen, 1991).

One of the most important steps in the production of cachaça, in order to guarantee the cachaça's quality is the distillation process, which can be conducted using two different apparatus: copper stills (artisanal cachaça) and stainless steel columns (industrial cachaça), as displayed in Fig. 11.2. The large-scale production of cachaça is characterized by the use of stainless steel columns, in which the distillation

Photo disclosure: *Santa Efigênia* distillery

Photo disclosure: Ypioca distillery

(A) (B)

Fig. 11.2 General picture showing a typical unit employed in the distillation process. Cooper alembics (A) and stainless steel columns (B). Part B: Photo by Felipe Jannuzzi. Used with permission from Mapa da Cachaça (http://www.mapadacachaca.com.br/).

process is continuous. In this case, the entrance of the fermented sugarcane juice (known as wine) inside the column and the respective exits of the distillate and the "*vinhoto*" happen simultaneously in different ways.

When the production is done in small production scale, the distillation process is generally carried out in copper alembics, where the separation of the distillate takes place in three different fractions through the so-called "cutting process." The first fraction to be collected is termed as "head," which removes the excess of more volatile and/or more soluble compounds in ethanol than in water. The second fraction is the "heart," the noble part of the distillate, used for commercial purposes. The last fraction, the "tail," contains excess of compounds less volatile than ethanol and more soluble in water than in ethanol, such as acetic acid and 5-HMF. The alcohol content in the distillate collected from the first cut (head fraction) reaches the range of 70.0%–55.0% (v/v) or a volume equivalent to 5%–10% of the total distillate volume. The second fraction, the heart, begins to be collected when the alcohol content reaches 55% (v/v) and ends up around 38% (v/v), which corresponds to 75%–80% of the total distillate volume. The last fraction, the tail, is collected when the alcohol content of distillate is below 38% (v/v), which corresponds to about 10% of the total distillate volume. The head and tail fractions are generally discarded or reused by some producers in a forthcoming distillation, added to a new wine.

The head fraction contains higher concentration of alcohols (total alcohols) when compared to the other fractions. Methanol, propanol, isobutanol, 1-butanol, and isoamyl alcohol are present in higher concentrations in the head fractions and isoamyl alcohol is the most abundant alcohol among the alcohols analyzed. The esters concentration is higher in the head fraction, being ethyl acetate the major ester compound present in the cachaças. Ethyl lactate has been found in higher concentrations in the tail and heart fractions than in the head fraction.

EC is more soluble in ethanol than in water and is mainly found in the head fraction. The median values found for EC concentrations were 65.0, 26.0, and 24.0 mgL^{-1} for the head, heart, and tail fractions, respectively. Thus, it is clear that a tuning in the first cut (head fraction) can reduce the urethane concentration in the distillate.

The acetic and lactic acid concentrations account for more than 90% of the total acidity of cachaças samples. Acetic, lactic, glycolic, succinic, and citramalic acids concentration, which are more soluble in water than in ethanol and with boiling points ranging from 112°C to 235°C, as expected, were higher in the tail fraction than in the others. Similar concentration of acetic acid in the heart and tail fraction point out the difficulty of removes the acidity through the cutting process.

Capric, lauric, myristic, and palmitic acids were predominant in the head fraction since they are more soluble in ethanol than in water and have boiling points ranging from 225°C to 270°C.

All the analyzed aldehydes, except 5-HMF and furfuraldehyde, are present in higher concentrations in the head fraction, being acetaldehyde the major compound. The 5-HMF and furfuraldehyde, which are more soluble in water than in ethanol, differently from the other aldehydes, present higher concentrations in the heart and tail fractions. Of the 1.7 billion L of cachaça produced annually in Brazil, ca. 30% are distilled in copper alembics. The Brazilian legislation does not differentiate cachaças according to their distillation processes, but applies different rates to the distillation processes. Thus, the cost per liter of the distillate makes the competition for the cachaça market unfavorable between the producers of alembic and column's cachaças. Studies have shown both distillates can be distinguished comparing their chemical profile differences using chemometrics tools (Reche et al., 2007). In this study, all the analyzed samples came from the distillation of different wines. Thus, the differences in chemical profiles between them could be attributed to the different procedures of the fermentation step to which the sugarcane juice was submitted, and not only to the distillation process.

In order to avoid the influence of the fermentative process in the chemical discrimination of both distillates, six different wines were distilled in both in cooper alembic and steel column. Thus, 24 distillates (6 for each alembic fractions—"*head, heart,* and *tail*," and others 6 columns distillates) were generated and their chemical composition compared. Analytical data were subjected to principal component analysis (PCA) allowing discrimination of four clusters according to the chemical profiles (Serafim et al., 2012).

Fingerprint monitoring of the cutting process in the alembic distillation, using comprehensive 2D GC and time-of-flight mass spectrometry (GC×GC/TOFMS), allowed the easy determination of the turning points of the process and high-resolution comparison of *cabeça* (head), *coracão* (heart), and *cauda* (tail) fractions (Cardeal et al., 2008). A fast "single shot" fingerprinting discrimination between alembics and columns cachaças was developed with success by the use of direct infusion electrospray ionization MS in the negative mode, ESI (−)-MS (De Souza et al., 2009a, b).

The material used in building of distiller apparatus in the sugarcane-spirit clearly influences its chemical profile. A quantitative chemical analysis of carboxylic acids, ketones, alcohols, aldehydes, esters, and dimethylsulfite (DMS) concentration in cachaças distilled from glass column packaged with copper, stainless steel, aluminum sponge, and porcelain balls were evaluated. The cachaças distilled in columns filled with copper or aluminum pot still present the lowest DMS contents and the higher sulfate and methanol contents. The cachaças from stainless steel or porcelain presented higher DMS concentration and lower sulfate ion and methanol concentrations (Cardoso et al., 2003a, b).

11.4.4 Aging Process

Maturation in oak barrels is an important widely spread practice in the production of alcoholic beverages. This process has been utilized for wine, Cognac, and whisky production in the Europe and North America. The contact of the beverage with the surface of wood species during the maturation process provides a diversity of chemical compounds (mainly phenolic compounds) that can continuously be extracted to the distillate, thus modifying its organoleptic properties. According to the Brazilian law, aged cachaça is the spirit matured in wood barrels, with maximum capacity of 700 L, during a minimum period of 1 year. The mechanism of aging process is based on the exchange of compounds present in wood species and the beverage, which can be classified in seven categories: direct extraction of wood compounds; decomposition of macromolecules; reaction between the wood compounds and the compounds of distilled beverage; interactions specifically involving the wood extract; reactions involving only the compounds present in the distilled; evaporation of volatile compounds through the cask surface; and formation of stable molecules (Piggott and Conner, 2003).

Due to its well-known and valuable sensorial characteristics, oaks barrels have been used extensively in the aging process for aged cachaças production. However, due to the wide Brazilian floral biodiversity, woods, such as cajarana (*Cabreala canjerana*), amburana (*Amburana cearensis*), bálsamo (*Myroxylon peruiferum*), amendoim (*Pterogyne nitens*), cabreúva vermelha (*M. peruiferum*), louro canela (*Ocotea fragantissima*), cerejeira (*Eugenia involucrata*), louro (*Cordia trichotoma*), pau d'arco (*Tabebuia vellosoi*), jatobá (*Hymenaea courbaril*), andiroba (*Carapa guianensis*), canela sassafraz (*Nectandra lanceolata*), cabreúva parda (*Myrocarpus frondosus*), pequi (*Caryocar brasiliense*), jatobá (*H. courbaril*), ipê (*Tabebuia* sp.), pereira (*Platycyamus regnellii*), freijó (*C. trichotoma*), goiabão (*Eugenia leitonii*), eucalipto (*Eucaliptus* sp.), itauba (*Mezilaurus itauba*), castanheira (*Bertholletia excelsa*), jequitiba (*Cariniana estrellensis*), andiroba (*C. guianensis*), balm (*M. peruiferum*), and pequi (*C. guianensis*) became the alternative to the cachaça aging. The use of a variety of wood species in the casks adds different sensorial properties to cachaça, with the production leaning for a regional preference. However, this practice has been developed over the years based in the local traditions and empirically without a proper evaluation of the dangers of extracts ingestion on consumer health. As for examples, the use of sassafras in the aging process, although it leads to the product appreciated for its sensorial qualities, provide to an increase of safrol concentrations, an essential oil, in the beverage, which according to the Food and Drug Administration (FDA) is considered a potential carcinogenic compound beside to present oxidant properties.

Considerable differences have been observed in the chemical profile of aged spirits in different woods species, which will depend on several factors, such as: type of wood, climate and soil, cooperage operations (wood cut, aging, and thermal treatment), aging time, and warehouse. The wood species presents a complex biological structure composed mainly of cellulose, hemicelluloses, and lignin, which interacting with the distilled by different ways, provides specific reactions during the extraction step. Cellulose is the major component of wood structure and the hemicelluloses can generate furfuraldehyde by hydrolysis. The degradation of lignin can generate coniferaldehyde, vanillin and vanillic acid, sinapaldehyde, syringaldehyde, and syringic acid concentrations (Piggott and Conner, 2003; Alcarde et al., 2014).

The chemical profile of volatile compounds and specific aging markers in cachaças aged for 36 months in casks made of 10 different types of wood such as amendoim, araruva, cabreúva, cerejeira, grápia, ipê roxo, jequitibá, jequitibá rosa, oak, and pereira was evaluated (Alcarde et al., 2010, 2014; Bortoletto and Alcarde, 2013). Sugarcane spirits aged in cerejeira, cabreúva, cerejeira, oak, and grápia casks presented high contents of total phenolic compounds. Cachaças aged in amendoim and jequitibá casks presented the highest values to volatile acidity and higher alcohols content. The cachaças aged in cerejeira, cabreúva, pereira, ipê roxo, and grápia casks presented below value for volatile congeners when compared with others wood species. Regarding to sinapaldehyde, syringaldehyde, syringic acid, coniferaldehyde, vanillin, vanillic acid, 5-HMF, furfural, and gallic acid congeners, the distillate aged in oak barrel presented the highest. The samples aged in jequitibá rosa also presented high sum of maturation-related congeners, followed by cerejeira (Alcarde et al., 2010, 2014; Bortoletto and Alcarde, 2013).

Gallic acid, syringaldehyde, and syringic acid were the characteristics compounds of spirits aged in oak cask. The vanillin concentration was the main compound present in the sugarcane spirit matured in jequitibá rosa and grápia (Alcarde et al., 2010, 2014; Bortoletto and Alcarde, 2013).

The differences in the chemical profile of cachaças aged in different wood species allied with chemometric tools have allowed a discrimination of distillates by its chemical characterization. A method for automatic recognition of the type of wood casks during the aging time, using information from a computer vision system, artificial neural networks, and *k-nearest neighbor* (k-NN) as algorithms for pattern recognition was developed. In this case, 100% of cachaças samples were for the problem correctly classified (Rodrigues et al., 2014, 2016).

The tannins' content of woods species can be differentiated in an indicator displacement assay (IDA) using peptide-based ternary sensing ensembles using a technique for fingerprinting the identity cachaças aged in five types of oak obtained from different countries, beside canela-sassafrás, balsamo, and amburana. This procedure recognized correctly 62.5% of the samples (Ghanem et al., 2017).

A simple, low-cost, and robust method to classify aged cachaças applying multivariate analyses in the UV-vis spectral data from wood extracts was used to identify Brazilian cachaças according to the wood species used in the maturation barrels. PCA and hierarchical cluster analysis (HCA) lead to identify six clusters of cachaças wood extracts from amburana, amendoim, bálsamo, castanheira, jatobá, and oak. Linear discriminant analysis (LDA) was used to classify with an accuracy of 90% correctly (Da Silva et al., 2012).

11.4.5 Geographical Traceability

The geographical origins of sugarcane spirits in Brazil are recognized by the producer's associations and by the Instituto Nacional da Propriedade Industrial (INPI) as geographic identification labels on the bottles. There is no scientific basis to support the adoption of these certification criteria. For wines, the geographic traceability is well known, structured, and organized, being based on their physical- chemical composition (mainly isotopic analysis) which provides the basis for territorial information ("terroir"). Different analytical methodologies such as electronic tongue and nose, liquid and GC, MS, and nuclear magnetic resonance, allied to chemometrics tools have been successfully used for this purpose (Serafim et al., 2016).

For distillates, information about geographical traceability is scarce in the literature. Tequila, for instance, is an alcoholic beverage made by fermentation and distillation of agave juice and the Mexican laws restrict its production. Samples produced in three different regions of the State of Jalisco, Mexico (Guadalajara, Tequila, and Amatitlan), were geographically identified by assessing their mineral content profiles (Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, S, Sr, and Zn), using discriminant analysis (DA) and support vector machines (Ceballos-Magaña et al., 2011).

Grape pomace distillates are distilled produced in most European countries wines producers. These beverages are traditionally produced in Galicia (NW Spain) from vinacce (skins, seeds, and stalks from the grapes). The European Union established the general regulations concerning the definition, denomination, and production of this alcoholic spirits and included Galicia as the only Spanish region with the permission of obtaining the geographic denomination for Orujo, as is permitted in the case in the same category as in the French (marcs), Italian (grappas), Portuguese (bagaceiras), Yugoslavia (Kommovica), Turkic (Raki), and in Greek (tsipouros). The volatile composition (alcohols, esters, aldehydes, and terpenes) has been employed to identify grape marc distillates (orujo) produced in three different regions of Spain (García-Martín et al., 2010; López-Vázquez et al., 2010). Brandies from France and Germany using Gram Schmidt orthogonalization were regionally identified evaluating their differences in the chemical profiles (Miyashita et al., 1989).

With respect to the cachaça's traceability, as have been observed its chemical profiles provide relevant information about the manufacturing practices, which can ensure the quality of the distillates. However, the chemical geographical characterization of the cachaças can be lacked due to theirs production conditions.

The Brazilian large territory extension provides differences in the edaphoclimatic factors, which can lead to different chemical and sensory profiles in the produced sugarcane spirits (De Orduña, 2010). The internal competition to produce a better quality product has improved the production procedures and intensified the merchandising of the products. At present, the geographical origins of drinks and foods have become a constant target of scientific research to ensure the authenticity and traceability of products to guarantee their quality.

Attempts to characterize geographically the Brazilian distillate were performed using its mineral contents (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Pb, S, Se, Si, Sn, Sr, and Zn). PCA shows a grouping of cachaças samples from Northeast, Central, and South regions. However, no successful separations were observed regarding to cachaças samples acquired from 15 different states of the Brazilian federation. Preliminary analyses accomplished attempting of classifying the samples according to their origin (15 classes) (Fernandes et al., 2005). These difficulties were already predictable since several variables of the cachaça production such as the soil composition, environmental conditions, and agrochemical treatments can contribute with the mineral content in the cachaças. The equipment used during the distillation process (cooper alembic or steel column) contributes to the cooper and lead concentrations in the distillate. Some difficulties in the geographical characterization of cachaças have been observed due to mistakes in the markers compounds. Benzo(a)pyrene, as already observed, is a PAHs used as a fine marker of harvested sugarcane burned and cannot be used in the geographical traceability.

Success was obtained in sugarcane spirits classification from selected regions of Brazil including São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Paraiba (PB), and Ceará (CE). The procedure utilized chemical data obtained for 24 compounds from 50 cachaças samples, produced using a similar procedure in order to avoid the influence of process in the results. Multivariate analysis was applied to the analytical results, and the predictive abilities of different classification methods were evaluated. PCA correctly identified five groups, and chemical similarities were observed between MG and SP cachaças samples and between RJ and PB distilled. CE samples presented a distinct chemical profile. Partial linear square DA (PLS-DA) classified 50.2% of the samples correctly, KNN 86%, and soft independent modeling of class analogy (SIMCA) 56.2%. Therefore, in this proof of concept demonstration, the proposed approach based on chemical data satisfactorily predicted the cachaças' geographic origins (Serafim et al., 2016).

The chemical typifycation of sugarcane spirits from Sao Paulo state, using 37 chemical compounds was evaluated in 81 cachaças samples produced in the following cities of São Paulo States: Águasde Lindóia, Arealva, Areias, Avaré, Bananal, Bariri, Batatais, Bernardino de Campos, Boa Esperança do Sul, Bonfim Paulista, Brotas, Caçapava, Cachoeira Paulista, Cafelândia, Cajuru, Cerqueira Cesar, Cerquilho, Charqueada, Cravinhos, Cruzeiro, Descalvado, Dois Corregos, Elias Fausto, Guará, Guarapiranga, Guararema, Itapira, Itápolis, Itatiba, Jarinu, Jaú, Leme, Lindóia, Monte Alegre do Sul, Nova Europa, Palmital, Pederneiras, Pindamonhangaba, Piracicaba, Pirassununga, Porto Feliz, Promissão, Ribeirão do Sul, Rio das Pedras, Salto de Pirapora, and Santa Adélia (Fig. 11.3).

Among these samples, 56 were distilled in cooper alembics, and the other 25 were distilled in steel columns. HCA and PCA were performed with both chemical data sets. A grouping of cachaças distilled in the steel column apparatus formed four specific clusters according to their chemical profiles, which correspond to analogous four distinct geographic regions of Sao Paulo state. Regarding to the cachaças distilled in copper alembics two clusters were identified; however, no correlation between their respective chemical similarities and geographical origins was observed. It is clear that the cachaças samples produce a "*terroir*" but it can be lost during the distillation process, mainly during the *cutting process* realized in the tail, heart, and head fractions separations (Serafim et al., 2012).

Fig. 11.3 Map displaying the cachaças' producers located at Sao Paulo state.

11.5 Conclusion

Frauds are very often perpetrated in high-value food commodities and those that are part of complex supply chains. Sugarcane spirits, cachaça, fit quite well these characteristics and are thus highly vulnerable. Cachaça traceability allows identify fraudulent products that can cause risks to the consumer health. The traceability of cachaças production steps has been achieved through a combination of qualitative and quantitative chemical analysis. The differences in the production process modify the chemical profile of the distillates attaching to the beverage a traceable "chemical fingerprint." As a result, the determination of the burned sugarcane crops, the fermentation, distillation, and aging process has been successfully evaluated. Although the large intrinsic variation of cachaças' production system is a factor that makes difficult the geographical traceability of distillates, this has become possible when some aspects, such as aging for instance, are considered.

An improvement in the sampling step, along with the inclusion of samples acquired from different production systems, together with a proper evaluation of additional chemical markers, can certainly lead to a more representative and robust predictive model for the chemical traceability of cachaças. All these together represent a relevant topic in the “food and beverages safety” arena, which is expected to continuously grow in the coming years.

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