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ETHYL CARBAMATE IN BRAZILIAN CACHAÇA

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ABSTRACT

Since suspicions were raised in the 1940s about the toxicity risks of ethyl carbamate (EC) ingested through fermented foods, tens of thousands of papers have been published, including research on precursor chemicals, formation mechanisms, analytical methods, toxicity (in *vitro* and in guinea pigs), levels of occurrence and prevention procedures. The IARC (2010) classified EC as category 2A (probably toxic) for the doses applied in the tests on guinea pigs, but found no evidence of carcinogenicity risks to humans from the ingestion of alcoholic beverages. The Codex Alimentarius (2020) recommends the monitoring of EC but does not propose the adoption of tolerance limits for its occurrence in fermented foods and beverages. In this work, based on the analysis of more than 5000 alembic cachaças, it was found that, consistently, over 90% of the samples kept the EC content below 400 µg EC/L. As alembic cachaças account for more than 95% of the brands of cachaça produced in Brazil, the authors propose that the limit of the current legislation, which is 210 µg EC/L, be increased to 400 µg EC/L.

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INTRODUCTION

Carbamates (or urethanes) are organic compounds endowed with a carboxyl bonded directly to an amine (Figure 1). Among them, ethyl carbamate (EC) - the ethyl ester of carbamic acid - had many applications in the production of medicines, foods, and cosmetics in the first half of the twentieth century. In the 1940s, however, the first warnings about EC toxicity risks emerged (Nettleship *et al.,* 1943). Since then their employment has declined until most applications have been discontinued (IARC, 2010). However, EC is still used today as an anesthetic in laboratory animals. In agriculture, carbamates still make up an important class of pesticides (IARC, 2010, Robler, 2014, Struger *et al.,* 2016).

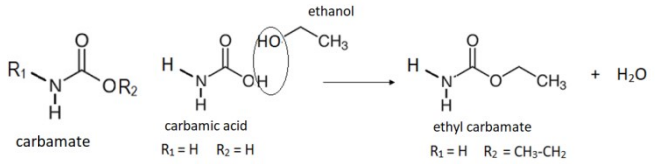

Figure 1. Carbamates and EC: carbamic acid ethyl ester

Questions about the spontaneous occurrence of EC in food began in the 1970s (IARC, 1974). In 1984, Canadian authorities mandated the monitoring of EC in fermented foods and beverages; since then, the measure has been followed by a limited number of countries (Ryu *et al.,*2015). Thousands of studies on the origins, toxicity, analytical methodologies, and options for controlling EC content in food and beverages have been developed in recent decades (Abt *et al.,* 2021, Jiao *et al.,*2014, Lachenmeyer *et al.,*2005, Weber & Sharipov, 2009). There is a consensus that it is a secondary compound inherent to the chemical nature of alcoholic fermentation musts. To a lesser extent, it can also occur in other foods (Denis *et al.,* 1997, Sen *et al.,*1993, Wu *et al.,* 2012). For cachaça, Brazilian legislation recently reaffirmed (MAPA, 2022) the tolerance limit of 210 µg/L that was establishedin 2014 (MAPA, 2014). The objective of this work is to gather subsidies for the evaluation of the assertiveness of this limit, considering the toxicity data and the production conditions of alembic cachaça, that is made in an agricultural environment and accounts for more than 95% of the brands of cachaça registered in Brazil.

LITERATURE REVIEW

In the context of fermented foods and beverages, it is clear that the origin of EC is multifactorial (Baffa, 2011; Battaglia *et al.,* 1990, Jiao *et al.,* 2014, Ohe, 2016). The formation occurs predominantly from

interactions of ethanol (the main product of alcoholic fermentation) with certain nitrogenous substrates (Baffa, 2011), especially urea, which can come from microbialmetabolism and agricultural practices (nitrogen fertilizer residues), as well as nutrients added to the wort to revigorate the yeast (although the addition of urea to the wort has been suppressed). Another important source is the cyanogenic glycosides of the raw material, which make up the defense mechanisms of plant physiology. To a lesser extent, EC can be formed in the absence of ethanol, from substrates endowed with double bonds and subjected to oxidation by the mechanism of free radicals (Aresta *et al.,* 2001; Moreira, 2019, Struger *et al.,* 2016).

Formation of EC by Microbial Metabolism: Indispensable to the metabolism of all living beings, nitrogen integrates the structures of amino acids, proteins, enzymes, nucleotides and nucleic acids (DNA and RNA), as well as vitamins such as biotin, thiamine, niacin, and riboflavin. It is part of the structure ofadenosine triphosphate, the main molecule that carries energy in the intracellular environment. Therefore, the activity of yeast necessarily presupposes a dynamic of nitrogen assimilation for vital functions and secretion of surplus levels. As a rule, it is disposed of in the form of urea (Kulaev *et al.,* 1985). In the specific case of alcoholic yeasts, the ethanol produced by the catabolism of sugar can react intracellularly with urea, forming EC. However, thisreaction is not advantageous for yeasts – and some strains have mechanisms to avoid it – because it forces the reprocessing of an ammonia molecule (Figure 2).

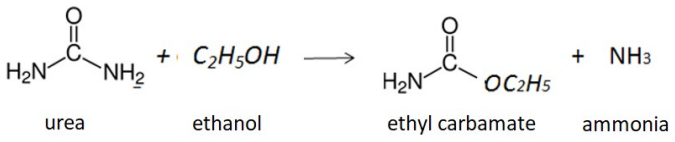

Figure 2. Direct reaction between urea and ethanol

Thus, most of the intracellular nitrogen surplus reaches the fermentation wort in the form of urea, where it can interact with ethanol – in varying proportions eventually favored by temperature (higher), pH (lower), oxygen availability (Berjarano *et al.,* 2015, Wu *et al.,* 2014). The presence of substrates such as carbamyl-phosphate, citrulline, ornithine and arginine, among others (Abt *et al.,* 2021, Orduña *et al.,* 2000, Stevens & Ough, 1993, Zhao *et al.,* 2013) also contributes, which can come from the raw material as well as from the extravasation of components of the urea cycle, by the lysis of dead cells (Figure 3).

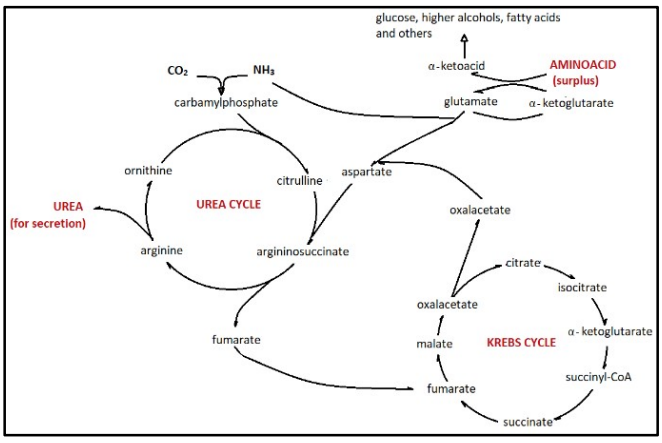

Figure 3. Schematic of the "urea cycle" for the secretion of surplus nitrogen from cellular metabolism and its integration with the Krebs cycle (respiratory, oxygen-dependent)

The availability of oxygen favors the secretion of urea, because the reactions that lead to its formation (Figure 3, left) are linked to the Krebs cycle (Figure 3, right), which only works in the presence of oxygen. In order tolimit the formation of EC, the suppression of free oxygen from the wort by the addition of potassium metabisulfite has been proposed (Hashiguchi *et al.,* 2010). However, under strict anaerobic conditions, microbial metabolism can be adjusted for direct ammonia secretion (Shalamitskiy *et al.,* 2023), with a deleterious effect on cell viability and to the detriment of metabolic pathways that lead to the formation of important components of the wort aroma, especially higher alcohols and esters (Maia *et al.,* 2020).

Formation of ec fromcyanogenic glycosides: Cyanogenic glycosides (GCs) are metabolites that make up the plant integrity defense system (Risk, 1990, Vetter, 2000). Many plants are GC producers. The structures and contents are different according to the plant species and, within each one, they can also vary according to the physiological and nutritional stage (Cravo *et al.,* 2019, Rosa Jr., 2005). Figure 4 illustrates the structures of durrin, found in sugarcane and grasses in general, amygdalin, found in stone fruits (such as peaches, cherries, plums, and apricots), and linamarin, found in cassava.

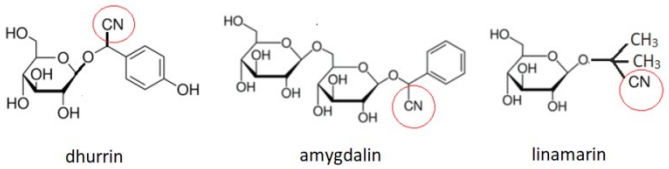

Figure 4. Structures of some cyanogenic glycosides

Faced with attack or threat from herbivorous insects and other predators, these substances enzymatically release hydrocyanic acid, which is volatile and highly toxic. And it can be admitted that certain procedures for harvesting, transporting and crushing sugarcane stalks have a sufficient predatory effect to activate the enzymatic release of cyanide, which can give rise to the formation of EC in fermentation musts, eventually on a scale as important as or even more than urea (Risk, 1990). The conversion of cyanide to urea is attributed to the mechanism illustrated in Figure 5. Cyanide interacts with the Cu^{+2} ion (commonly found in fermented beverages), forming cupric cyanide, which breaks down into cuprous cyanide (CuCN) and cyanogen (C_2N_2). The oxidation of cyanogen results in cyanide and cyanate, the latter of which reacts with ethanol forming ethyl carbamate (Weber & Sharypov, 2009).

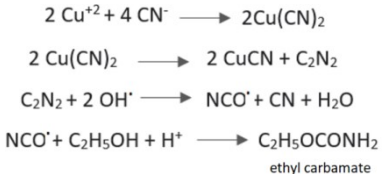

Figure 5. Formation of EC from cyanide catalyzed by Cu^{+2} and oxygen (free radicals)

The importance of the GCs as EC precursors depends on the physiological specificities of each raw material. Generally, this route is much less prominent in cachaça and other sugarcane-derived beverages than in tiquira and stone fruit spirits (Lachenmeyer *et al.,* 2005; Voldřich & Kyzlink, 2006).

Formation of EC from Unsaturated Compounds: Aresta *et al.* (2001) proposed the mechanism of EC formation without the direct participation of ethanol, through to the oxidative effect of UV radiation on the double bonds of unsaturated compounds (Figure 6). It is a less prominent alternative route, possibly more relevant in baking, due to the higher content of unsaturated fatty acids.

EC Formation in Beverage Storage: In the presence of the chemical precursors, EC formation continues during the storage of the beverage, whether fermented or distilled (Kodama *et al*., 1994). Berjarano *et al*. (2015), for example, reported an increase from 6 µg EC/L (after fermentation) to 120 µg EC/L during the storage period of wines in oak barrels.

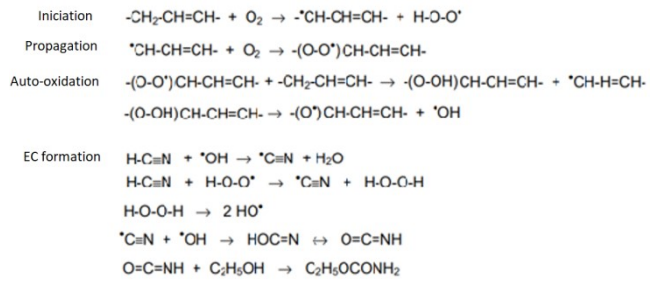

Figure 6. EC formation from cyanide and double bonds in carbon chains

By the stoichiometry of the reaction between urea and ethanol, it is possible to estimate the potential for EC formation during storage, based on the residual urea content, as well as the EC content already formed, based on the ammonia content, as shown in Table 1.

Table 1. Estimation of EC formation potential in sugarcane spirits

	Values in µg/L			
	Urea (*)	CE to be formed	Ammonia (*)	CE already formed
Minimum	180	267	0	0
Maximum	73200	108580	648	3204
Average	18960	28124	234	1157

(*) Urea and ammonia data source: Polastro *et al.* (2001)

In Table 1, the high levels of ammonia and urea can be attributed to the fact that, at the time of the analyses (2001), there was still no legislation on the EC content of distilled beverages in Brazil. During storage, EC formation is exponentially catalyzed by ambient temperature. In fact, several authors have reported success in mitigating EC by performing both fermentation and subsequent storage of wines at low temperature (Hasnip *et al*., 2004, Larcher *et al., 2013*). Wu *et al.* (2014), seeking to optimize the production process of yellow rice wine, which includes heat treatment, monitored the evolution of EC content during production and storage. The authors reported a slow evolution during fermentation, which became accentuated in the course of heating for sterilization. Subsequently, the rapid cooling allowed to maintain the EC content at a lower level than the wine submitted to natural cooling. In particular, storage temperature (for 400 days) was the factor that most affected EC content (Figure 7).

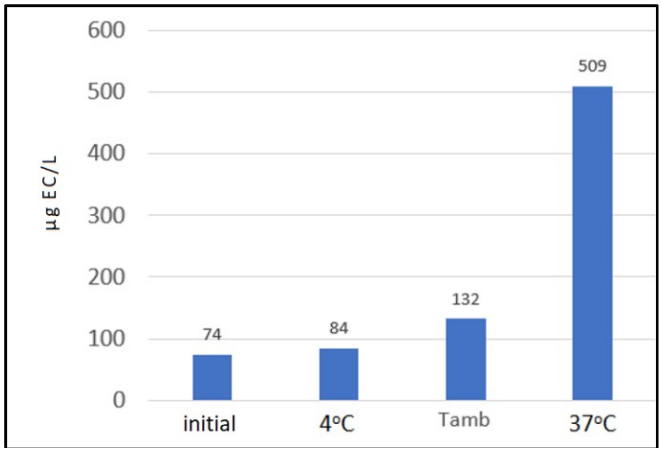

Figure 7. Evolution of EC content in stored yellow rice wine at different temperatures for 400 days (Data source: Wu *et al*., 2014)

Light also catalyzes the formation of EC, especially through UV radiation, which potentiates the formation of free radicals (Abt *et al*., 2021; Zacaroni *et al*., 2015). By way of illustration, Lachenmeier *et al*. (2005) reported a mean increase of 1,300 µg/L after 4 hours of direct exposure of stone fruit spirits to the sun.

EC Metabolism: It has been shown that EC is rapidly and evenly distributed throughout the human body through the bloodstream, regardless of the route of administration, which can be oral, dermal, subcutaneous or intraperitoneal. The catabolism occurs mainly in the liver, where most of it (90%) is converted into ethanol, ammonia, and carbon dioxide. A fraction of less than 0.5% is enzymatically hydrolyzed to vinyl carbamate and an even smaller fraction, around 0.1%, is converted to N-hydroxycarbamate (Forket & Lee, 1997; Guengerich and Kim, 1991; Miller &Miller 1983, Weber & Sharypov, 2009). The rest is secreted without metabolic alteration. There is a limit to metabolic capacity, already estimated at 4.8 mg/kg weight for male rats and above 48 mg/kg weight for laboratory mice (Nomeir *et al.,* 1989; O'Flaherty & Sichak, 1983). Above the limit, EC can remain longer in the body.

Toxicity and Mutagenicity: For acute toxicity, doses estimated at between 70 and 170 g of EC would be required (NIOSH, 1985). Therefore, the risks of toxicity are assessed for the cumulative effect of continuous ingestion. Researched in a wide range of microorganisms, no risks of mutagenic effects in bacteria were found. In eukaryotic cells, positive effects have been reported from high and different doses according to the genetic peculiarities of each organism. Generally, carcinogenic effects have been attributed to the level of occurrence of vinyl carbamate and N-hydroxycarbamate adducts with RNA, which can be specific for each kind of organism (Park *et al*, 1993; Sakano *et al*., 2002). Therefore, according to Schlatter & Lutz (1990), it is very difficult or even impossible to extrapolate mutagenicity data between different organisms. In 2010, the results of thousands of in *vitro* and in *vivo* studies (with guinea pigs) were evaluated by IARC, an agency located in Lyon (France) that coordinates research and scientific investigation at an international level, with the aim of contributing to the implementation of effective policies for cancer prevention. The studies focused on both the toxic effects of ethanol and EC, and the full report is available on the internet (IARC, 2010). Once the work was concluded, the following summary was issued:

> "The Working Group reviewed the epidemiological evidence on the possible associationbetween alcohol consumption and cancer in 27 anatomical locations, and reaffirmed the previous conclusion (...) that the upper digestive tract (oral cavity, pharynx, larynx, phage) and liver diseases are causally related to alcohol consumption. In addition, the Working Group considered that there is sufficient evidence to conclude that cancer and female breast cancer is also on this list. With regard to ethyl carbamate, the Working Group concludes thatthere is insufficient evidence in humans of the carcinogenicity of ethyl carbamate. However, there is sufficient evidence in experimental animals of the carcinogenicity of ethyl carbamate, vinyl carbamate and vinyl carbamate oxide."

Importantly, the above considerations refer to the risks of ingesting EC (and also ethanol) through alcoholic beverages. It must be remembered that, as commodities, several "carbamates" still find technological applications. In particular, the class of pesticides referred to as "carbamates" includes the pesticide Aldicarb, popularly referred to as "chumbinho". In agricultural practice, there are numerous reports of contamination of soil and waterways, with human poisoning, deaths of animals and aquatic life, as well as suicide attempts (Blacker *et al*., 2010; Grendon *et al*., 1994; Risher *et al*., 1987; Sengler *et al.,* 2000; Silberman *et al*., 2020; Xavier *et al*., 2007).

Legislation: In most countries of the world there are no restrictions on the EC content of natural occurrence in alcoholic beverages. The Codex Alimentarius (2020) recommends monitoring this parameter, but does not stipulate a tolerance limit. Among the countries that regulate EC content, the limits established for distilled beverages range from 125 to 1000 µg/L. For cachaça, Brazilian authorities established the limit of 210 µg EC/L (Table 2).

Table 2. EC limits set for distilled spirits in various countries

Beverage	Alcohol content % vol.	Bound µg/L	Countries
Stone fruit spirits	40 - 50	400	Canada and the Czech Republic
		800	Germany
		1000	France
		1000	European Union
Distilled in general		125	USA
		150	Canada, Czech Republic, France
		210	Brazil

Table 3. Options for combating EC in alcoholic beverages

Where/Way	How	Source
Raw material	Correct selection, application and management of fertilizers	Silva, 2019
	Selection of varieties with lower cyanogenic glycoside content	Russo & Reggiani, 2014 Cravo et al., 2019
Fermentation	Low Temperature Low nitrogen content	Weber & Sharypov (2009)
Enzymatic Degradation	Acid urease - to degrade urea in wort	Andrich et al., 2009 Fidaleo et al., 2006 Kobashi et al., 1988 Miyagawa et al., 1999 Suzuki et al. 1979 Yang et al., 2010
	Urethanase – to hydrolyze ethyl carbamate	Masaki, 2022 Guengerich & Kim, 1991
Genetic engineering	Microorganisms, genetically modified to inhibit the urea cycle or induce urethanase production	Dong et al., 2022 Grossmann et al., 2011 Guo et al., 2016 Jung et al., 2022 Kulaev et al., 1985 Larcher et al., 2013
Wort additives	Metabisulfite as an antioxidant	Hashiguchi et al., 2010
	Diammonium phosphate to reduce amino acid catabolism	Adams & van Vuuren, 2010
	Phenolic antioxidants	Jiao et al., 2014
Distillation	Adjustments in the distillation fractions of sugarcane spirit Do not reuse head spirits Bi-distillation of cachaça	Bruno et al., 2007 Labanca, 2009
Packaging	Low temperature Restriction to any exposure to sunlight	Hasnip et al., 2004 Wu et al., 2012
Adjuvants in storage	Activated carbon (EC adsorption) Ion Exchange Resins	Abt et al., 2021 Shalamitskiy et al., 2023 Park et al., 2009 Bruno, 2006

Levels of occurrence and preventive/corrective measures: The highest EC contents occur in distilled beverages produced from stone fruits and cassava, due to the high GC contents in the raw materials. Lachenmeier *et al*. (2005) found levels of up to 18000 µg/L (average of 1,400 µg/L) when analyzing 631 samples of stone fruit spirits inGermany. A similar mean (1197 µg/L) was reported by Diachenko *et al*. (1992) regarding the analysis of 89 samples in the United States. In Brazil, tiquira analyses showed levels of up to 3500 µg/L, which were mainly attributed to the linamarin present in cassava (Lachenmeier *et al*., 2010). The current levels of occurrence in alembic cachaça will be seen in section 4. Since the 1990s, producers have relied on manuals for EC prevention infermented and distilled beverages (Butzke & Bisson, 1997). Subsequently, and until now, new options have been studied and proposed (Table 3). In the field of genetic engineering, research points to the possibility of repressing the catabolism of amino acids and the urea cycle, through depletion of the RNA fractions that encode the respective enzymes (Coulon *et al*., 2006; Grossman *et al*., 2011; Guo *et al*., 2016; Jung *et al*., 2022; Vigentini *et al*., 2017; Wu *et al*., 2016). Genetic regulation for intracellular production of urethanases has also been proposed (Dong *et al*., 2022). However, these studies do not include evaluations of changes in the metabolism of the secondary components that determine the sensory quality of the beverage. For alembic cachaça, as the production is based on successive batches of fermentation of the fresh broth with reuse of the yeast (pé-de-cuba), it has already been shown that the ferment incorporates yeasts from the environment, which ones, after two to three weeks, can quantitatively surpass the selected strains (Alvarenga, 2019; Badotti, 2010; Santos, 2013). Some authors (as Kobashi *et al*., 1988 and Fidaleo *et al*., 2006) proposed the use of acid urease to hydrolyze the urea in the wort, releasing ammonia and carbon dioxideThe proposal is interesting, but it is important to consider that ureases are nickel-dependent metalloenzymes. Furthermore, accessibility and costs are impediments to routine application in an agricultural environment. The application of urethanases, or EC-hydrolases, also has been proposed. These enzymes have already been identified in several bacteria, yeasts, and filamentous fungi (Masaki, 2022). However, they are not yet commercially available. Even when they, in the future, may be available, their employment in alcoholic beverages production may be also difficulted by issues of accessibility and financial cost. In sequence, the EC contents in "alembic cachaça" are presented. It is important to point out that this drink is manufactured by thousands of producers, in an agricultural environment and on a scale predominantly below 300 L per day. It is highly valued for the care

given to each stage of the production process and for its sensorial quality, distinguishing itself sharply from cachaça produced on an industrial scale, which can reach 300,000 and even 1,000,000 liters per day.

MATERIAL AND METHODS

Cachaça samples: EC levels were surveyed in 5539 samples of alembic cachaça from several Brazilian states, analyzed from 2015 to 2023 at the LABM Laboratory (ISO 17025 certified). In this set, there are both freshly distilled and matured and/or aged cachaça. EC dosage: in all samples, EC was quantified in a gas chromatograph (Agilent GC 4350A) coupled to a 7036A mass detector, equipped with an automatic G4513 A injector, operating in electronic impact mode with 70 eV and m/z 62 selective ion monitoring. HP-FFAP polar phase capillary column (50m x 0.20mm x 0.33µm. Helium carrier gas (purity: 99.999%), flow rate of 1.5 mL/min; initial temperature 90°C (2 min), followed by rise to high up to 240°C at the rate of 10.7°C/min). Gun and detector temperature 230°C. Injection volume 2 µL (direct injection). Throughout the analysis period, there was a progressive reduction in the minimum limit of quantification. For standardization purposes, values below 50 µg/L were composed of a single group.

RESULTS AND DISCUSSION

The analytical results were organized by year of analysis and by alcohol content range (Table 4).

Table 4. EC content in samples of alembic cachaça analyzed from 2015 to 2023

Year	EC content (µg/L)							Number of samples
	< 50	50-200	200-400	400-600	600-800	800-1000	> 1000	
2015	43	70	14	4	2	2	0	135
2016	125	70	10	3	1	0	1	210
2017	119	201	56	8	3	4	3	394
2018	175	187	140	7	2	1	0	512
2019	277	190	61	14	4	2	0	548
2020	273	342	172	37	10	2	2	838
2021	320	401	162	14	12	4	4	917
2022	361	428	139	25	10	2	4	969
2023	334	433	185	33	15	2	14	1016
Total	2027	2322	939	145	59	19	28	5539
%	36,6	41,9	17,0	2,6	1,1	0,3	0,5	...

It can be observed that there was a progressive increase in the number of samples analyzed, since the publication of IN 28 (MAPA, 2014) when the limit of 210 µg EC/L came into force. In the average of the whole period, the EC content was below 50 µg/L in 36.6% of the samples, below 200 µg/L in 78.5% of the samples (36.6% + 41.9%). Asignificant percentage was in the range of 200 to 400 µg/L (17%, corresponding to 939 samples analyzed). A total of 251 samples (4.5%) had values above 400 µg/L; in 28 samples (0.5%) the content was above 1000 µg/L. Therefore, EC contents in the alembic cachaça were located predominantly in the range of 0 (n.d.) to 400 µg/L. It is important to note that, in several cases (and at the request of the respective producers), the same cachaça was successively analyzed after storage for six months to up to three years. In general, initial values below 50 or 200 µg/L showed a progressive increase, almost always to the 200- 400 µg/L range, eventually exceeding 400 µg/L. In addition, according to the analysis of different harvests of the same producer, in several cases there were marked changes according to the year of production (data not shown).There are some suspicion that these fluctuations may also be related to the processing of sugarcane from different geographical origin in each harvest. Figure 8 shows the percentages of samples with levels below 210 µg/L (limit of current legislation) and below 400 µg/L. It should be noted that between 2015 and 2019, more than 80% of the samples analyzed had EC content below 210 µg/L, thus meeting the limit of the current legislation. From 2020 onwards, there was a decrease in the percentage of compliance with this limit, which may be related to multiple factors, including climatic variations (Gobbo-Neto & Lopes, 2007).

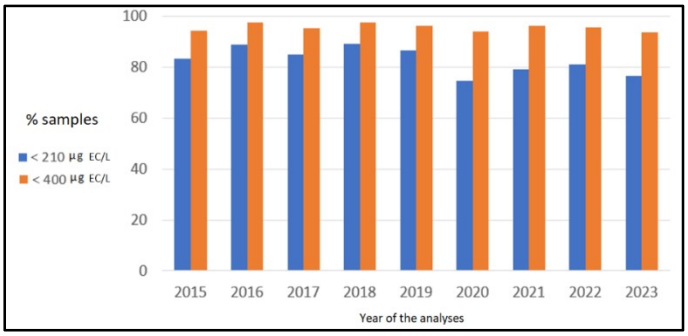

Figure 8. Percentage of samples analyzed from 2015 to 2023, with EC contents below 210 µg/L and below 400 µg/L

At the limit of 400 µg/L, however, the results were uniform, always above 90% of the samples analyzed. The fact that EC levels in the range of 210 to 400 µg/L characterize non-compliance with current legislation penalizes a large number of producers who already make their best efforts in favor of the quality of their beverages. In particular, it represents an obstacle for many of them who have invested in conquering the foreign market. As already mentioned, most countries do not set limits for EC content and several countries allow limits of up to 1000 µg/L in distilled spirits. However, the trade norm is that the beverages to be exported comply with the legislation of the country of origin. As for the risk of carcinogenic effect of EC – it is necessary to understand that, after thousands of studies, the questions and suspicions raised from the 1940s onwards have not been confirmed, as was effectively attested by the IARC (2010). Even assuming the limit of 1000 µg EC/L, the potential carcinogenicity of EC ingested through distilled beverages is meaningless when compared to the potential toxicity/carcinogenicity of alcohol abuse (Guo & Ren, 2010).

Comparing limitations to ethanol and EC consumption, it must be recognized that:

- For ethanol, there is a level of consumption recognized as healthy, conducive to improved cardiovascular health andincreased longevity compared to abstainers (Maraldi *et al*., 2009; Maskarinec *et al*., 1998).
- Above the healthy limit, ethanol consumption, in addition to being potentially carcinogenic (EFSA, 2007; IARC, 2010) has been proven to be associated with numerous devastating diseases, such as heart disease, Alzheimer's disease, stroke, liver disease, and diabetes mellitus (Baliunas *et al*., 2009; George & Figueiredo, 2010; Maskarinec *et al*., 1998; Mocan *et al*., 2008; Ohkubo *et al*., 2009; Osana, 2009).
- For EC, there is no range of intake that is beneficial to health. But any limit on the intake of cachaça that can be linked to the carcinogenic potential of the EC content with 400 µg/L will markedly exceed the most abusive limits on alcohol consumption. By way of illustration, for an adult weighing 60 kg, ingesting 1 mg EC/kg weight would require the consumption of 150 liters of the drink containing 400 µg EC/L. This dose, however, would be completely insignificant for the effect of acute toxicity. The chance of chronic toxicity would only arise after repeating the experience, at least, for many days.

CONCLUSION

There is not a single report of human cancer that has been attributed to the ingestion of EC through food and drink. By understanding the available data it is possible to desmytify the role of EC as a potentially carcinogenic agent in the field of distilled beverages and, in particular, Brazilian cachaça. We believe that producers would benefit from the increase of the EC limit in cachaça to 400 µg/L, without any demerit to good production practices, without prejudice

to the quality of the beverage and without risk to the health of consumers.

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