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Stochastic Approach to Estimating Heavy Metals (Cu, Pb, Cd, Fe) and Methanol in Artisanal Beverages

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Abstract

"Koutoukou" is an alcoholic drink consumed in several West African countries, particularly in Ivory Coast. In most cases it is made in an artisanal way. Given the quality of the material used in manufacturing and therefore likely to contain harmful substances, the objective of this study is to determine the contents of certain heavy metals and methanol (disease risk factors) then to estimate the exposure risk using the probabilistic Monte Carlo method. The results obtained indicated high concentrations of several chemical contaminants such as iron (21.010-66.247 mg/L), copper (3.778-47.931 mg/L), lead (6.395-21.446 mg/L), cadmium (3.451-7.712 mg/L) and methanol (34.679-1781.983 mg/L). The levels of these compounds were found in most samples to be higher than the various standards applicable to alcoholic beverages. Also, the Monte Carlo method revealed that the risk of exposure to these toxic molecules was very high for consumers. It is: 96.9%; 98.7%; 95.8%; 99.3% and 61.7% respectively for Cd, Pb, Cu, Fe, and Methanol.

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Keywords

Risk analysis, heavy metals, methanol, Monte Carlo.

Introduction

"Koutoukou" is a spirit produced in Ivory Coast. It was introduced from Ghana around 1940 (Camara, 2002). It is classified in groups 4 and 5 of edible brandies (Yao, 2009). It is obtained by distillation of wine from different palm trees including oil palm, raffia and palm tree or even sugar water with yeast added (Koffi *et al.,* 2017). In Côte d'Ivoire, the proliferation of oil palm plantations encouraged by the State and the aging of these plantations (Cheyns *et al.,* 2001) have contributed to the large part of the use of palm wine for the production of koutoukou. Called "akpeteshie" in Ghana, "sodabi" in Benin and Togo and "odontol" in Cameroon (Camara, 2002), koutoukou is sold in Ivory Coast in very specific places, generally in neighborhoods where people live very low income. This alcohol is proudly placed alongside other liqueurs at receptions or other social events (Akoa, 2004). It is used during ancestor worship ceremonies (rituals and libations), during funerals, weddings and also in the preparation of traditional therapeutic beverages (Yao, 2009). Served dry or diluted, it sometimes breaks the consumption record during these ceremonies (Akoa, 2004).

In Ivory Coast, its production and consumption were banned in 1964, however "koutoukou" continued to be manufactured clandestinely in practically all regions (Camara, 2002). Faced with the scale of this clandestine manufacturing, the Government ended up authorizing its official manufacturing in 1999 by imposing new safety rules on producers (Camara, 2002). Despite all the restrictions imposed, its manufacturing remains traditional with the use of artisanal materials (AIP, 2015). Thus, the work of Akoa (2004) noted that this artisanal brandy contains significant quantities of methanol which is a toxic product and harmful to health (Yao, 2009). Studies carried out by Koffi *et al.,* (2019), showed the existence of heavy metals (lead, copper and iron) and methanol in this drink. In addition, cases of death have been noted following the consumption of this drink, particularly in the town of Bouake in Ivory Coast. According to Koffi *et al.,* (2019), "koutoukou" producers are not trained in good hygiene and especially manufacturing practices, and this could therefore constitute a real danger for the health of the consumer (Dibi *et al.,* 2017). Thus, this study aims to estimate the risk of exposure to toxic molecules present in this drink using the Monte Carlo method with the aim of preventing consumer health.

Specifically, this will involve:

- Determine the physicochemical parameters
- Quantify toxic molecules (Cd, Cu, Fe, Pb and methanol)
- Estimate the level of exposure risk using the probabilistic Monte Carlo method

Materials and Methods

The biological material for the present study consists of samples of "koutoukou" alcohol purchased from sellers in the localities of Bonoua, Aboisso and Abengourou.

Sampling

Thirty (30) bottles each containing one (1) liter of "koutoukou" were purchased from traders (retailers) from the three study areas: Bonoua, Aboisso and Abengourou. For a total of ninety (90) samples. These samples were transported to the laboratory in aseptic conditions limiting any external contamination for the various analyzes according to the modified AFNOR method (1994).

Determination of alcohol content

The alcoholic strength by volume (TAV) was determined using a GAY-Lussac alcoholometer according to the method of OIV (2012). Thus, 0.5 L of each sample was transferred into a graduated cylinder. The alcoholometer was immersed in the liquid in order to determine the alcohol level and the TAV reading was made directly on the graduated rod of the device.

Determination of pH

The determination of the pH of the different samples was carried out using a pH meter according to the method (OIV, 2012). Two hundred and fifty (250) mL of each sample was poured into beakers and the electrode of the pH meter (previously calibrated) was immersed in the liquids. The pH reading of the drink is determined on the pH meter screen. This operation is repeated three times.

Determination of total titratable acidity

Total acidity was measured using the method described by the International Organization of Vine and Wine (OIV, 2012). It consisted of introducing 25 mL of sample into a 500 mL conical bottle using a pipette.

In this flask, 200 mL of distilled water and 3 drops of the mixed indicator solution (0.1 g of indigo carmine and 0.1 g of phenol red dissolved in 40 mL of water and make up to 100 mL with ethanol). Then, the mixture was titrated with the 0.05 M sodium hydroxide solution until the yellowish-green color changed to purple.

Determination of heavy metals

The quantification of heavy metals Fe, Pb, Ca and Cu was carried out by atomic absorption spectroscopy (AAS) in accordance with IITA methods (1981). The binary mixture for the analysis used consisted of compressed air and acetylene.

The detection was made using four (04) lamps corresponding to the mineral to be quantified (spectrAA Varian, No. 808.1024). Before the analyses, the atomic absorption spectrometer was calibrated with a standard solution (Atomic Spectroscopy Standard 21). The dosage was done by adding 5 mL of hydrogen peroxide (H_2O_2)

and 1.5 mL of nitric acid (HNO_3) to a beaker containing a mass of 0.3 to 0.5 g of sample.

The mixture was heated to 60°C until it boiled and evaporated on a sand bath. Then 50 mL of distilled water was added before reading. The concentrations of the different metals are calculated using the following expression:

$$T\ (mg/L) = ((C_{éch} - C_{blanc})/m) \times V \quad (1)$$

Eq (1)

Whith:

$C_{éch}$: Sample concentration (mg/L)

C_{blanc}: Concentration of the blank test (mg/L)

m: Weight of butter (g)

V: Volume of distilled H_2O added

Determination of methanol

The determination of methanol was carried out according to the OIVMA-AS312-03A method and modified (OIV, 2012). The elements found were expressed in milligrams per liter (mg/L).

Evaluation at exhibition

The koutoukou after manufacture is not subject to any treatment, it is put on sale where it will be consumed directly. The quantity ingested (I) of toxic substance in this scenario was established by a relationship between the quantity of drink consumed and the quantity of chemical contaminant determined (Assidjo *et al.,* 2013). Ingestion (I) is therefore the product of the quantity of drink consumed (***Q***) by the quantity of chemical contaminant (***C***).

$$I = Q \times C \quad \text{Eq (2)}$$

Statistical analyzes

Statistical analyzes were carried out using Statistica 7.1 software for adjustment to distribution laws (normal, uniform and exponential). Using the Matlab R2016a software, a calculation program, developed and implemented, was used to carry out the probabilistic estimation of exposure risk using the Monte Carlo method (Griva *et al.,* 2009; Abouo *et al.,* 2023).

Results and Discussion

Samples taken in three areas (Bonoua, Aboisso and Abengourou) were tested for heavy metals (Fe, Cu, Cd and Pb) in "koutoukou". Table I shows the quantities and significant differences of heavy metals and methanol present. Iron appeared in all samples, with very high values: 37.83mg/L in Aboisso; 25.08mg/L in Abengourou and 45.41mg/L in Bonoua. Cadmium levels were 6.63 mg/L in Bonoua, 5.52 mg/L in Abengourou and 4.66 mg/L in Aboisso. Copper levels are higher in samples from Aboisso and Bonoua, at 31.08mg/L and 28.35mg/L respectively. As for lead, it is higher in samples from Abengourou and Bonoua: 15.27 mg/L and 11.20 mg/L. The analysis of Table I shows that all the samples taken have a quantity of cadmium higher than the standard which is 0.2 mg/L. Adjusting the data on the quantities of alcohol (to be consumed) to the laws of probability (normal, exponential and uniform) makes it possible to obtain tables numbered II to VI. When analyzing Table II, the lowest parameter values $\chi 2(0.873)$ are observed for the uniform distribution with the highest probability (0.954). For almost all of the samples taken, the quantity of copper is greater than the 1 mg/L standard. The evaluation of the parameters of adjustment to the three (03) distribution laws used for the study observed in Table III shows that the values of the parameters $\chi 2$ smallest (1.224) and largest probability (0.878) are observed at the level of the exponential law. Lead levels exceeded the 0.2 mg/L standard for all samples. The experimental data on the quantities of beverages consumed were fitted to the distribution laws shown in Table IV, the analysis of which indicates a higher probability value (5.680) and a lower $\chi 2(0.908)$ for the exponential distribution. The samples taken show iron levels above the norm (0.2 mg/L). Analysis of the fit to the three (3) distribution laws used for the study shows that the lowest $\chi 2$ (4.142) and highest probability (0.911) values are observed for the exponential distribution, which is also shown in Table V for cadmium. Table VI shows the methanol content of "koutoukou" samples taken in the different zones. Methanol appeared in all samples analyzed, with higher levels in Aboisso and Abengourou, with values of 1731.4 mg/L and 70.17 mg/L respectively. All the samples taken had methanol levels above the standard 500 mg/L (Table I). Experimental data on the quantities of beverages consumed were fitted to the distributions shown in Table VI, the analysis of which indicates a higher probability value (0.901) and a lower $\chi 2(6.863)$ for the uniform distribution. Table VII shows the physico-chemical parameters analyzed in the samples taken from the various zones. These are hydrogen potential (pH), titratable acidity (TA) and alcoholic strength by volume. The pH of the samples ranged from 3.66 to 4.69. It was recorded at 3.66 in Aboisso, 3.87 in Bonoua and 4.69 in Abengourou. Bonoua and Aboisso have the highest titratable acidity values, at 1522.16 mEq/L and 1170.06 mEq/L respectively, while Abengourou has the lowest at 212.05 mEq/L. TAV values are 40% at Aboisso, 30% at Abengourou and 45% at Bonoua.

The critical limit for the amount of cadmium permitted in "koutoukou" is 0.2 mg/L, set by the International Organization of Vine and Wine (OIV). As for the cumulative probability density curve in Figure 2, it shows a corresponding probability of 1-0.031=0.969; i.e. 0.969 ≈ 96.9%. The cadmium levels in the various samples taken are outside the tolerance limit, not fit for consumption and present too high a risk..According to the OIV, the critical limit for the amount of copper permitted in "koutoukou" is 1 mg/L.

By analyzing the cumulative probability density curve observed in figure 3, the corresponding probability is 1-0.042=0.958 or 0.958 ≈ 95.8%. The copper levels in the various samples taken are outside the tolerance limit, and therefore non-compliant and present too high a risk..For lead, the critical limit of the authorized quantity is 0.2 mg/L admitted for spirits, liqueur and brandy according to the Quebec Alcohol Society (QAS). Observation of the cumulative probability density curve in Figure 4 shows that the corresponding probability is 1-0.013=0.987; i.e. 0.987 ≈ 98.7%. The lead levels in the various samples taken are out of bounds, not fit for consumption and also present a sufficiently high risk. The critical limit for iron is 0.2 mg/L, as defined by the European Union (EU). By analyzing the cumulative probability density curve (figure 5), the corresponding probability is 1-0.007=0.987; or 0.993 ≈ 99.3%.

The iron levels in the various beverage samples taken are outside the tolerance limit and are not suitable for consumption, and the risk incurred is practically equal to 100%. Ivorian standards set the critical limit for the permitted amount of methanol at 500 mg/L in alcoholic beverages. Analysis of the cumulative probability density curve presented in figure 6, shows that the corresponding probability is 1-0.383=0.617; i.e. 0.617 ≈ 61.7%.

The methanol levels of the various "koutoukou" samples are beyond the tolerance limit, and are non-compliant for consumption and also present high risks after consumption.

The results of analyses carried out on the various "koutoukou" samples from the three study areas (Bonoua, Aboisso and Abengourou) revealed the presence of heavy metals and methanol. Heavy metals are naturally occurring elements with atomic numbers and densities five times higher than the density of water (Izah *et al.,* 2016). Heavy metals are classified into two groups: essential and non-essential heavy metals.

Essential heavy metals play an important role in human health, while non-essential heavy metals can be toxic to the body when the concentration exceeds the tolerable limit (Woyessa *et al.,* 2015). Iron is generally present in the environment, and its composition varies according to soil composition. The quantities of iron found in the various samples are above the limit defined by the European Union, which is 0.2mg/L (EU, 1998). The concentrations recorded could be explained by the fact that this beverage is produced in metal barrels that are heated to high temperatures (Koffi *et al.,* 2019). The possible degradation of the barrels during heating could be at the origin of the high presence of this metal in the beverage produced. These results are in line with the work of Koffi *et al.,* (2019), who found high iron concentrations of between 22.23 and 42.50mg /L in "koutoukou". Cadmium is a heavy metal that is toxic to human tissue, even in low concentrations. This mineral occurs naturally in the environment as well as in many industrial products (Ubuoh, 2013). All samples analyzed contained cadmium (5.52 mg/L; 4.66 mg/L and 6.63 mg/L). These various quantities of cadmium present in the samples are well above the permissible limit (0.2mg/L) set by the OIV (OIV, 2012). This high quantity could be due to the use of artisanal equipment, also by soil and pesticide contamination (Koffi *et al.,* 2019). These results are similar to those obtained by Koffi *et al.,* (2019), who found values between 5.06 mg/L and 5.44 mg/L. Lead is emitted by anthropogenic activities such as fossil burning, paint production and bacterial production (Izah *et al.,* 2016). The samples analyzed contained quantities of lead (9.96 mg/L; 15.27 mg/L and 11.20 mg/L) in excess of the 0.2 mg/L standard allowed for spirits, brandy according to the Quebec Alcohol Society (QAS). The presence of lead in the samples would be justified by contamination of the raw material during storage in the barrels during handling prior to production (Koffi *et al.,* 2019). The lead levels in this study are higher than those in the work of Woldemariam and Chandravanshi (2011), who reported lead concentrations above the standard (6.31 mg/L) in traditionally manufactured imported beverages purchased in supermarkets in Addis Ababa, Ethiopia.

Table.1 Average values for toxic molecules (metals and methanol)

Parameters	*Aboisso*	*Abengourou*	*Bonoua*	*Standards (TRV (mg/L)*
Cd (mg/L)	4.660 ±0.047	5.520 ±0.063	6.630 ±0.074	**0.200**
Cu (mg/L)	31.082 ±0.795	11.930 ±0.486	28.350 ±0.673	**1.000**
Pb (mg/L)	9.96 ±0.198	15.270 ±0.386	11.200 ±0.374	**0.200**
Fe (mg/L)	37.830 ±0.504	25.080 ±0.228	45.410 ±0.845	**0.200**
Methanol	1731.400±3.163	1399.43±2.437	70.170±0.087	**500.000**

TRV : Toxicological reference values (OIV, 2012)

Table.2 Parameters for fitting to Cu distributions

Distribution laws	χ^2	*p*
Normal	31.562	0.000
Uniform	**0.873**	**0.954**
Exponential	42.261	0.001

Table.3 Parameters for fitting to Pb distributions

Distribution laws	χ^2	*p*
Normal	100.501	0.000
Uniform	233.281	0.000
Exponential	**1.224**	**0.878**

Table.4 Parameters for fitting to Fe distributions

Distribution laws	χ^2	*p*
Normal	125.919	0.000
Uniform	153.571	0.000
Exponential	**5.680**	**0.908**

Table.5 Parameters for fitting to Cd distributions

Distribution laws	χ^2	*p*
Normal	84.753	0.000
Uniform	109.324	0.000
Exponential	**04.142**	**0.911**

Table.6 Parameters for adjustment to methanol distributions

Distribution laws	χ^2	*p*
Normal	113.983	0.000
Uniform	92.694	0.000
Exponential	**6.863**	**0.901**

Table.7 Physico-chemical parameters present in the samples

Parameters	Aboisso	Abengourou	Bonoua
pH	3.660 ±0.015	4.690 ±0.008	3.87 0±0.017
TA (mEq/L)	1170.060 ±12.111	212.050 ±4.112	1556.660 ±17.450
TAV (%)	40 ±0.001	30 ± 0.011	45 ±0.009

Figure.1 Sample of "koutoukou"

Figure.2 Cumulative probability density curve for Cd

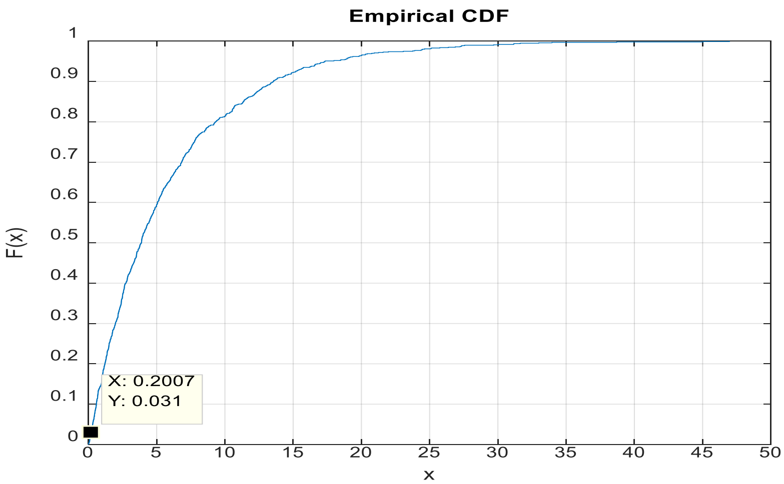


Figure.3 Cumulative probability density curve for Cu

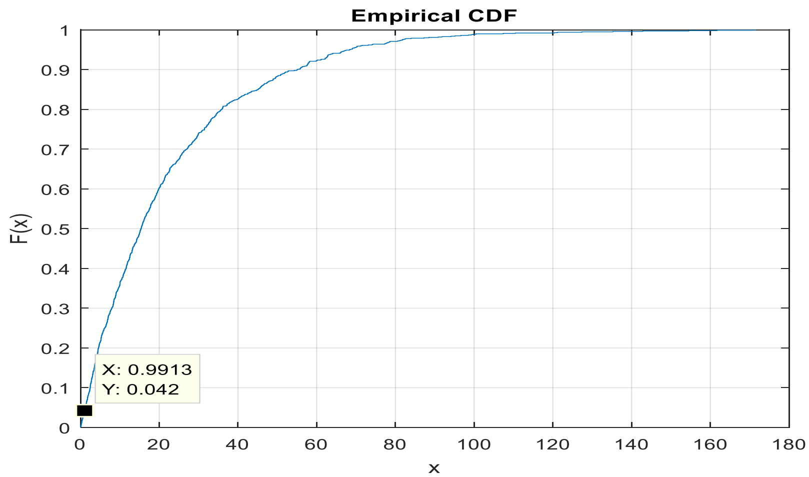


Figure.4 Cumulative probability density curve for Pb

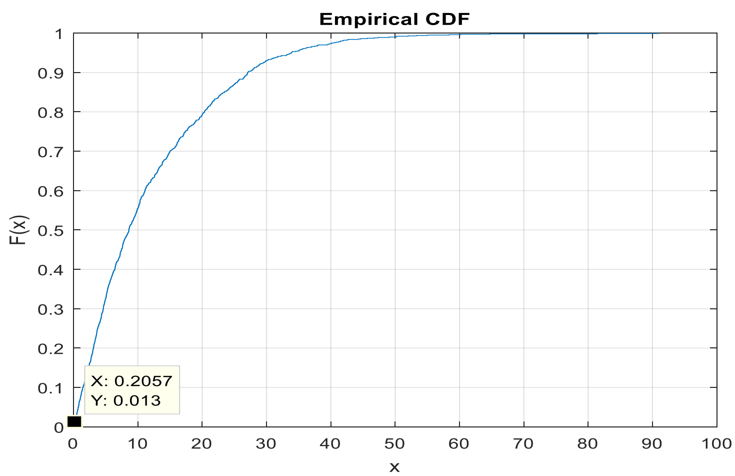


Figure.5 Cumulative probability density curve for Fe

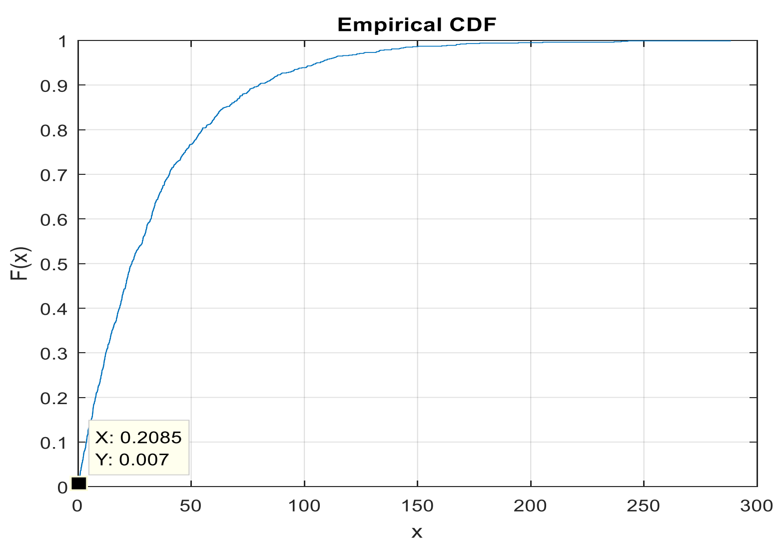
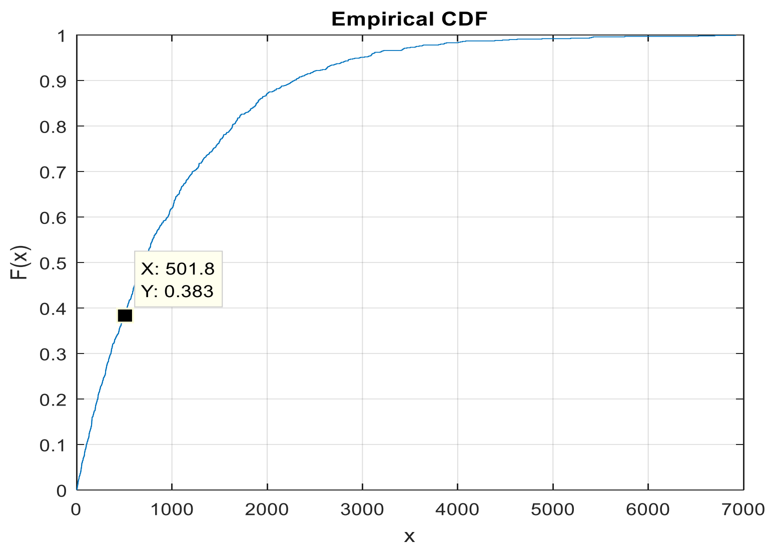


Figure.6 Cumulative probability density curve for methanol



As for copper, one of the essential heavy metals present in the environment (water and soil), its high presence in the samples, with various quantities (31.082 mg/L; 11.93 mg/L and 28.85 mg/L) well above the 1 mg/L limit set by the International Organization of Vine and Wine (OIV, 2012). These high quantities could be explained by the fact that distillation equipment consisting of copper pipes subjected to high temperatures would degrade over the course of production (Koffi *et al.,* 2019). These results are much lower than the findings of Iweala *et al.,* (2014), who found copper concentrations of between 0.02 and 3.55 mg/kg in traditional herbal alcoholic beverages. The methanol values obtained from the samples are respectively 1737.4 mg/L; 1399.43 mg/L and 70.17 mg/L for the localities of Aboisso, Abengourou and Bonoua. For two localities, they exceed the 500 mg/l limit set by the Ivorian standard for alcoholic beverages. Lack of control over the distillation process is thought to be the cause of these high methanol concentrations. Indeed, producers have no means of temperature control capable of selecting precise distillation ranges and types of alcohol (Akoa, 2004). The pH of the various beverages obtained is acidic, which could be due to the activity of microorganisms (yeasts) during fermentation. The pH of palm wine decreases with increasing fermentation time (Nwanchukwu *et al.,* 2006). Our results are in line with those of Koffi *et al.,* (2019), who obtained pH values ranging from 3.33 to 4.17 in their work. In terms of alcoholic strength by volume, the values obtained are 40% for Aboisso, 30% for Abengourou and 45% for Bonoua. With regard to the analysis results, the alcohol content of over 35% ethanol justifies their classification as class 4 and 5 brandies.

Conclusion

The aim of this study was to estimate the risk of exposure to toxic molecules in "koutoukou". The results showed high concentrations of several chemical contaminants, including heavy metals (iron, copper, lead, cadmium) and methanol. The levels of these compounds were well above the various standards applicable to alcoholic beverages. As a result, the Monte Carlo method revealed that the risk of exposure to these toxic molecules was very high for consumers. It is: 96.9; 98.7; 95.8; 99.3 and 61.7% respectively for: Cd, Pb, Cu, Fe, and Methanol respectively.

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