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ARTICLE

Analysis of Methanol and Ethanol Content in Illegal Alcoholic Beverages using Headspace Gas Chromatography: *Case Studies at Rwanda Forensic Institute*

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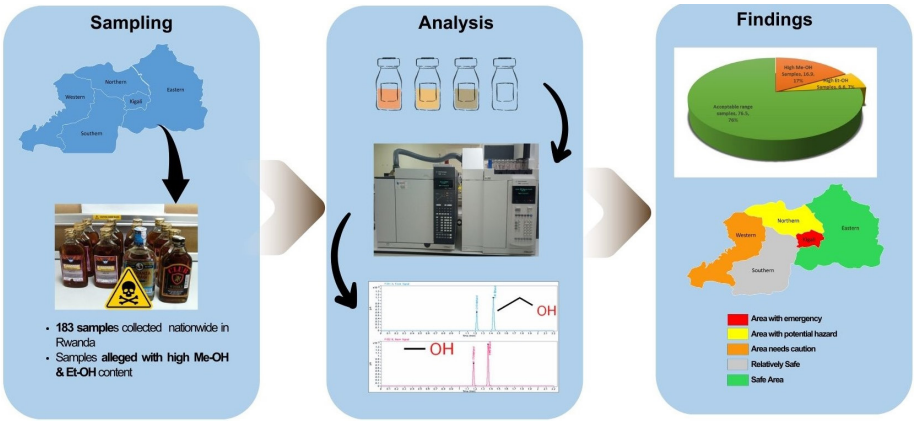

Illegal alcoholic production in Rwanda has become a critical social concern due to its widespread prevalence and associated health risks, stemming from the inexpensive distillation process employed by illicit producers. This often results in the unintentional production of toxic methanol instead of safe ethyl alcohol, presenting a substantial hazard in the context of illegal alcohol production. Methanol, when consumed, transforms into toxic formic acid in the body, causing severe health complications by disrupting mitochondrial respiration. Between 2021 and 2022, 183 forensic cases related to methanol and ethanol poisoning were collected nationwide and examined at the Rwanda Forensic Institute (RFI). Utilizing Headspace Gas Chromatography, the study aimed to quantitatively assess the extent of the problem and compare results to Rwanda's allowable limits for methanol and ethanol in alcoholic beverages. The analysis demonstrated a significant variation in ethanol content (3.8% to 98.9% v v^{-1}) and methanol levels (32% to 58.3% v v^{-1}), with 6.6% of samples exceeding the methanol limit (0.5% v v^{-1}) and 16.9% surpassing the ethanol limit (45% v v^{-1}). The City of Kigali emerged as the primary contributor to non-compliance, notably associated with specific brands like K'bamba, African Buffalo, Merry Cane, Royal Castle, and unbranded alcoholic beverages. Importantly, none of the samples tested positive for both methanol and ethanol simultaneously, emphasizing the urgency of monitoring and regulating Rwanda's alcoholic beverages market to ensure compliance with acceptable methanol and ethanol levels and safeguard public health.

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INTRODUCTION

Alcohols, characterised by the presence of a hydroxyl (-OH) group, are a group of hydrocarbons with the potential for toxicity when consumed excessively, leading to intoxication and organ damage. Among the clinically significant toxic alcohols, methanol and ethylene glycol stand out.[1] With historical use in preserving fluids in ancient Egypt, methanol was first identified in 1661 when it was distilled from boxwood and termed the spirit of the box. Later, in 1834, Dumas and Peligot determined its molecular makeup, coining the term, "methylene" from Greek roots meaning "wood wine." Industrial manufacturing of methanol and its derivatives began in 1923.[2]

Illicit alcoholic beverages, which are often produced using industrial methylated spirits and local fermentation processes, can pose risks due to their potentially high levels of methanol.[3] Factors influencing methanol production in fermented alcoholic beverages included raw material characteristics, sterilisation temperatures, pectin content, and pectin methyl esterase (PME) activity,[4,5] with microbes also contributing to methanol production.[6,7]

Although methanol by itself is not very harmful, the hepatic alcohol dehydrogenase (ADH) in the liver breaks it down into formaldehyde, which is then transformed into toxic formic acid in the body. This highly toxic compound can cause serious health problems by interfering with mitochondrial respiration.[8-11] In the production of fruit spirits, the degradation of pectic substances by naturally occurring enzymes like PME leads to significant methanol formation, with the concentration varying based on fruit type and enzyme-substrate interaction.[8] Methanol is rapidly absorbed through various routes and is distributed in tissues with higher lipid or water content, primarily in the eyes, muscles, blood, gastrointestinal tract, liver, and cerebrospinal fluid.[12] Its metabolism mainly occurs in the liver by alcohol dehydrogenase, leading to the production of toxic metabolites.[13,14]

Exposure to methanol can result in symptoms such as headache, vertigo, fatigue, nausea, vomiting, blurred vision, permanent blindness, and even death due to limited detoxification capacity for formic acid.[1,15,16] Methanol is rapidly absorbed with close to 100% bioavailability and a distribution similar to total body water.[17,18]

Ethanol inhibits methanol metabolism by competing for alcohol dehydrogenase, and a blood ethanol level above 100 mg dL^{-1} effectively halts methanol catabolism, leading to its persistence in the body.[19] If ethanol and methanol are ingested together, methanol remains in the body until most of the ethanol is metabolized.[19] However, detecting and quantifying methanol and ethanol accurately in alcoholic beverages suspected of containing excessive amounts is essential in criminal investigation and community safety.

Based on 183 forensic cases that Rwanda Forensic Institute received from 2021 and 2022, with a focus on methanol and ethanol poisoning cases, the study aimed to shed light on the extent of methanol and ethanol toxicity, identifying the source and prevalence of methanol contamination in illicit alcoholic beverages in Rwanda. This was done by using the Headspace Gas Chromatography Flame Ionization Detector (HS-GC-FID). The study findings can guide public health initiatives, law enforcement operations, and educational campaigns aimed at lowering the production and consumption of illicit alcoholic beverages and promoting safer alternatives.

MATERIALS AND METHODS

Reagents and samples

Every substance utilized in this study met the stringent criteria for analytical gas chromatography reagent quality. We acquired certified reference material (CRM) for ethanol and methanol (100 mg dL-1 in water, Cerilliant®, Germany). The Milli-Q water filtration system (Merck Millipore) provided the ultra-pure water. We procured high-purity (5.5 purity) helium, hydrogen, and dried air gases suitable for GC from Gas Labs Limited (Nairobi, Kenya).

A total of 183 samples of illegal alcoholic beverages were collected from various parts of the country, including the City of Kigali (comprising 80 samples), the northern province (consisting of 61 samples), the southern province (with 22 samples), the western province (accounting for 11 samples), and the eastern province (including 9 samples).

Based on their original labels, these samples were divided into different brands, including K'bamba with 22 samples, Muriture with 17 samples, African Buffalo with 14 samples, African Gin with 11 samples, Rabiant with 11 samples, Royal Castle with 9 samples, Merry Cane with 4 samples, and unbranded alcoholic samples with a total of 95 samples. The collection of these samples spanned the years 2021 to 2022 in response to suspected incidents of methanol poisoning, which tragically resulted in 15 fatalities and a multitude of other severe health complications including permanent blindness. The distilled alcoholic samples were maintained at a constant room temperature (RT), whereas the fermented alcoholic samples were carefully stored under refrigerated conditions to ensure the prevention of any further changes or fluctuations in the ethanol and methanol content within the samples.

Rwanda Investigation Bureau (RIB) collected and transported these samples to the laboratory for forensic examination. Every collected sample was properly packed, labelled, and with a unique identification number. To uphold ethical considerations, the identities of the shops and manufacturing factory owners, from which these samples were collected, were kept confidential throughout the study.

Preparation of calibration standards

The analytical standards solutions for methanol and ethanol were prepared using the gravimetric-volumetric approach. We used a process where the analytes were progressively added to the deionized water to prevent the analytes' evaporation.

Each standard was separately added to a 10 mL volumetric flask after being carefully weighted, and then diluted using ultra-pure water to the calibration point. The flasks were promptly sealed, gently inverted and homogenized several times until the standards were evenly distributed throughout the solution. Additionally, a 6.0 mL amount of standard mixture was transferred into a 20 mL headspace vial and sealed using headspace crimp aluminium caps equipped with PTFE silicon septum. This sealing was done immediately, followed by the subsequent analysis using HS-GC-FID. It is worth noting that calibration standards were always freshly prepared for this purpose. A calibration curve, consisting of six data points 0.01 g%, 0.05 g%, 0.1 g%, 0.2 g%, 0.5 g%, and 1 g% (w v^{-1}), for both methanol and ethanol, was generated by plotting the concentrations of the analytes standards against their respective responses (peak areas), as illustrated in Figure 2.

Preparation of sample solutions

Using ultra-pure water, sample solutions were generated using various dilution factors (1:100 for distilled alcoholic samples and 1:25 for fermented alcoholic samples). To obtain the distilled samples, 100 µL of the sample was taken out and placed in a 10 mL volumetric flask that was then filled to the meniscus with ultra-pure water. While 400 µL of the sample was drawn and put into a 10 mL flask of the same type, and then topped off to the meniscus, in the case of fermented samples. A 6.0 mL quantity of each type of sample was taken, put into a 20 mL headspace vial, and sealed with headspace crimp aluminium caps that had PTFE silicon septum. This sealing was completed quickly, and then the instrument analysis came next.

Instrumentation

All measurements were made with Gas Chromatography (GC)-Flame Ionization Detector (FID) (Agilent Technologies Inc., Model 7890B GC) equipped with Headspace (HS) sampler (Agilent Technologies Inc., model 7697A HS). Headspace vial (20 mL) and Headspace Crimp Aluminium caps, PTFE/Silicon septum (Agilent Technologies Inc., US) were used to prepare sample solutions. OpenLab ChemStation, an instrument software, was employed to analyse the data.

Table I. HS-GC-FID conditions

GC-FID conditions	
Carrier gas	Helium, 4 mL min^{-1}
Detector	FID1 & FID2, 300 ºC
Detector gas	Hydrogen 25 mL min^{-1}; Air 300 mL min^{-1}; Nitrogen 10 mL min^{-1}
Injector	Split/split less type; 250 ºC; Split 25:1; split flow 100 mL min^{-1}; inlet pressure 17.382 psi; Septum purge flow 3 mL min^{-1}
Chromatographic column	DB-ALC1 (Agilent Technologies Inc., 123-9134); 20 ºC – 260 ºC (280 ºC), 30 m * 320 µm * 1.8 µm; In: Front SS inlet He; Out: Front Detector FID; Flow rate 4 mL min^{-1}; Pressure flow 17.382 psi DB-ALC2 (Agilent Technologies Inc., 123-9234); 20 ºC – 260 ºC (280 ºC), 30 m * 320 µm * 1.2 µm; In: Front SS inlet He; Out: Back Detector FID; Flow rate 4 mL min^{-1}; Pressure flow 17.382 psi
Temperature program	Set point (initial): 60 ºC; hold time 2.2 min; Post run: 60 ºC
Headspace sampler parameters	
Oven temperature (ºC)	60
Loop temperature (ºC)	70
Transfer line temperature (ºC)	80
Transfer line type	Fused Silica
Transfer line diameter (mm)	0.53
Vial equilibration (min)	5
Vial pressurisation gas	Nitrogen
Loop size (mL)	1
Vial standby flow (mL min^{-1})	20
Sample amount (mL)	6
Injection duration (min)	0.5
GC cycle time (min)	0.4
Vial size (mL)	20
Vial shaking	Level 1, 18 shakes min^{-1}
Fill pressure (psi)	15
Extraction mode	single
Post injection purge	100 mL min^{-1} for 1 min

Validation parameters

The analysis was performed using a method that had already been developed and validated in the laboratory settings, following established standard guidelines for routine analysis.[20]

The chromatographic profiles of both methanol and ethanol appeared to be satisfactory, as depicted in Figure 1.

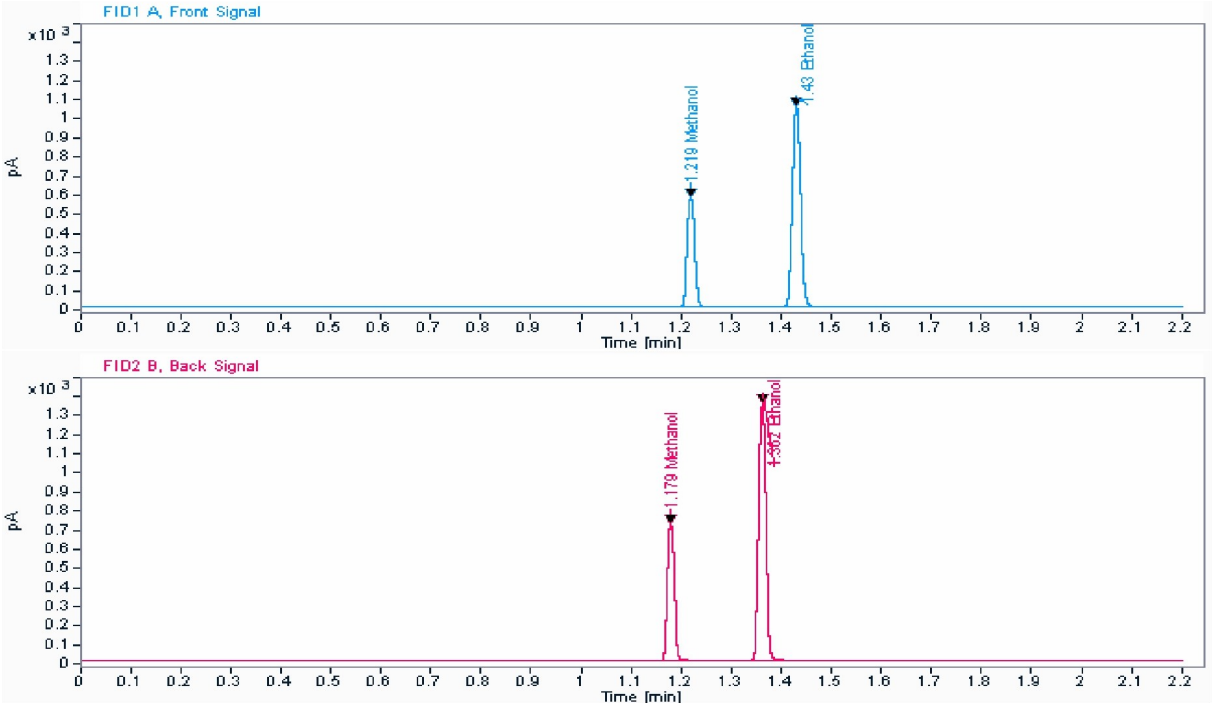

Figure 1. Chromatograms of ethanol and methanol at 0.5 g% using two detectors (FID1 A & FID2 B).

Calibration Model and Carryover

Calibration standards were set up, covering a range of concentrations from 0.01 g% to 1 g% w v^{-1}) for both methanol and ethanol in standard aqueous solutions. Six calibration points achieved a significant correlation (R^2) of 0.997 for methanol and 0.998 for ethanol, indicating a linear connection between the calibration curves. The method used a linear regression model for calibration as shown in Figure 2.

Following each calibrator, an examination of the blank samples was conducted to assess the carryover at different concentrations. It was observed that within the concentration range of 0.01 g% to 1 g%, no carryover was detected in any of the blank samples that followed the calibrators, for ethanol and methanol.

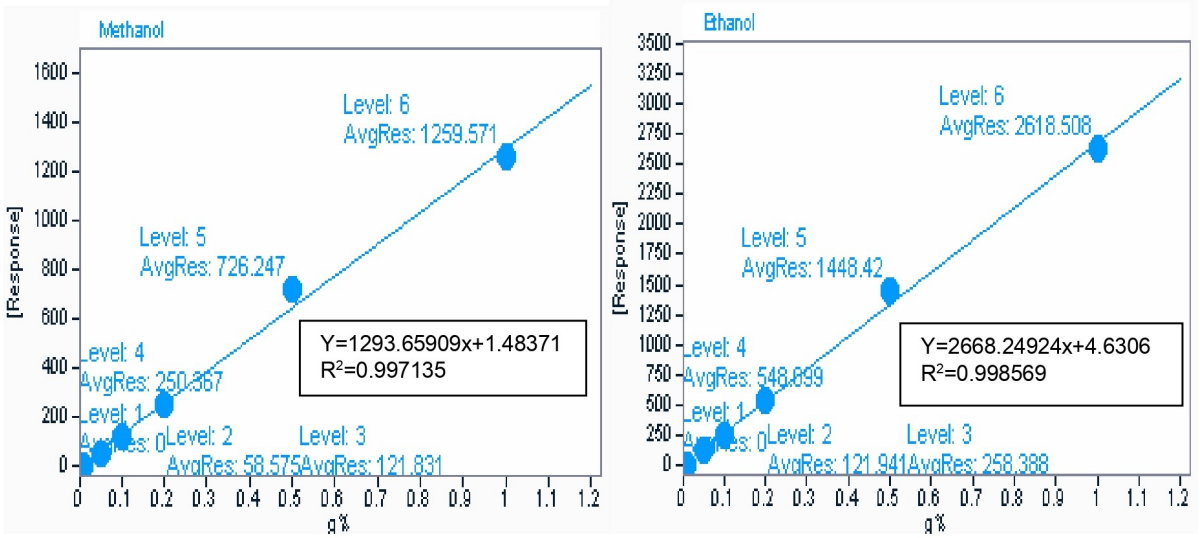

Figure 2. Calibration curves for methanol and ethanol utilising the HS-GC-FID.

Determination of quantification and detection limits

The lower limit of quantification (LOQ) for both analytes was 0.03 g% (w v^{-1}) (signal-to-noise=10), whereas the lower limit of detection (LOD) was 0.01 g% (w v^{-1}) (signal-to-noise=3), as shown in Table II.

Precision and accuracy

Precision was evaluated by conducting analyses in triplicate of three quality control samples (QC) with concentrations of 0.05 g% (low QC), 0.5 g% (medium QC), and 0.8 g% (high QC) for all analytes over five days, along with a freshly prepared calibration curve. The coefficients of variation (% CV) for methanol were determined to be 10.1%, 6.5%, and 5.0%, respectively. Accuracy was also assessed, resulting in values of 114%, 92%, and 100.8% for the low, medium, and high-control concentration samples, respectively. For ethanol, the coefficients of variation (% CV) were determined to be 8.2%, 5.6%, and 3.4%, respectively. Accuracy was also assessed, resulting in values of 105%, 96.8%, and 100.2% for the low, medium, and high-concentration control samples, respectively as indicated in Table II.

Table II. Method validation parameters and calculated values

Analyte	Interference	Linear Range (g%) and R^2	Linear regression equation	Limits (g%)		Carryover	Overall Accuracy (%CV)			Overall Precision (%RSD)		
				LOD	LOQ		Low QC (0.05)	Med. QC (0.5)	High QC (0.8)	Low QC (0.05)	Med. QC (0.5)	High QC (0.8)
Me-OH	None	0.01-1 0.997	Y=1293.65909x+1.48371	0.01	0.03	None	114	92	100.8	10.1	6.5	5.0
Et-OH	None	0.01-1 0.998	Y=2668.24924x+ 4.63046	0.01	0.03	None	105	96.8	100.2	8.2	5.6	3.4

Interference studies

In the interference studies, the method consisted of analysing spiked control samples to assess the potential interference caused by a variety of substances, including acetic acid, ethyl acetate, acetone, propanol, isobutanol, butanol, acetaldehyde, and an array of other volatile compounds that could be present in alcohol beverages. After these spiked samples were analysed, no interference peaks attributable to methanol and ethanol were seen during the retention time (t_R).

RESULTS AND DISCUSSION

Analytical results

The quantification of methanol and ethanol in samples being analysed was established using peak areas as a fundamental measurement parameter.[21] In the process of quantitatively analysing analytes, a linear regression model was employed. As the concentration of the analyte increased, the analysis response (peak area) also increased in a linear manner.[22,23]

Every country sets safety guidelines that determine the allowable percentage of alcohol by volume (ABV) for the production and sale of alcoholic beverages.[19,24] In Rwanda, the permissible limits for methanol and ethanol are less than 0.5% and 45% (v v^{-1}), respectively, in alcoholic beverage production and sale.[25]

The measurements of ethanol and methanol concentration in each sample were first calculated in mass percentage (g% w v^{-1}) during analysis, and the results were subsequently converted into alcohol by volume (ABV) or volume percentage (% v v^{-1}).

This observation underscored the significance of ensuring that alcoholic beverages adhere to safety regulations to safeguard public health and legal compliance. Table III offers a comprehensive illustration of the levels of methanol and ethanol detected and quantified in the samples. It is important to note that the ethanol content showed substantial variation, ranging from 3.8% to 98.9% (v v^{-1}) in the samples containing ethanol, and between 32% and 58.3% (v v^{-1}) for the samples in which methanol was detected. The figures marked in red denote instances where the concentrations of methanol or ethanol exceeded the acceptable limits defined by Rwanda's Ministry of Health, leading to legal proceedings.

Table III. Methanol and ethanol concentrations (% v v^{-1}) in analysed samples (2021-2022) and sample collection details, with red-highlighted values indicating levels exceeding Rwanda Ministry of Health limits and subsequent legal consequences

Case No.	Sample Brand	Me-OH(%vv^{1})	Et-OH(%vv^{-1})	Collection Area	Case No.	Sample Brand	Me-OH (%vv^{-1})	Et-OH (%vv^{-1})	Collection Area
1	Unbranded	ND	37.1	North	29	Unbranded	ND	43	North
2	Unbranded	ND	36.9	North	30	African Gin	ND	37	North
3	Unbranded	ND	30.8	North	31	African Gin	ND	42.8	North
4	Unbranded	ND	32.8	North	32	African Gin	ND	34.2	North
5	Unbranded	ND	28	North	33	Unbranded	ND	43.4	North
6	Unbranded	ND	30.4	North	34	Unbranded	ND	29.7	North
7	Unbranded	ND	31.9	North	35	Unbranded	ND	37.3	North
8	Unbranded	ND	29.6	North	36	African gin	ND	50	North
9	Unbranded	ND	21.8	North	37	Unbranded	ND	50.7	North
10	Unbranded	ND	23.4	North	38	Unbranded	ND	44.8	North
11	Unbranded	ND	30.3	North	39	Unbranded	ND	39.6	North
12	Unbranded	ND	35.6	North	40	Unbranded	ND	40	North
13	Unbranded	ND	40	North	41	Unbranded	ND	43.6	North
14	Unbranded	ND	43	North	42	Unbranded	ND	21	North
15	Unbranded	ND	47.8	North	43	Unbranded	ND	48.6	North
16	Unbranded	ND	38.9	North	44	Unbranded	ND	43.7	North
17	Unbranded	ND	35.6	North	45	Unbranded	ND	46.7	North
18	Unbranded	ND	38.9	North	46	Muriture	ND	12.5	North
19	Unbranded	ND	39.4	North	47	Unbranded	ND	47.6	North
20	Unbranded	ND	37.1	North	48	Unbranded	ND	41.9	North
21	Unbranded	ND	38	North	49	Unbranded	ND	39.6	North
22	Unbranded	ND	39.7	North	50	Unbranded	ND	47.2	North
23	Unbranded	ND	37.4	North	51	Unbranded	ND	41.6	North
24	Unbranded	ND	44.4	North	52	Unbranded	ND	43.3	North
25	Muriture	ND	4.9	North	53	Unbranded	ND	44.1	North
26	Unbranded	ND	44.1	North	54	Unbranded	ND	44.8	North
27	Unbranded	ND	46.9	North	55	Unbranded	ND	13.5	North
28	Unbranded	ND	49.4	North	56	Unbranded	ND	41.2	North

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Table III. Methanol and ethanol concentrations (% v v^{-1}) in analysed samples (2021-2022) and sample collection details, with red-highlighted values indicating levels exceeding Rwanda Ministry of Health limits and subsequent legal consequences (continuation)

Case No.	Sample Brand	Me-OH(%vv^{1})	Et-OH(%vv^{-1})	Collection Area	Case No.	Sample Brand	Me-OH (%vv^{-1})	Et-OH (%vv^{-1})	Collection Area
57	Unbranded	ND	32.3	North	85	Royal castle	ND	47.7	Kigali
58	Unbranded	ND	44.6	North	86	Royal castle	ND	50.1	Kigali
59	Muriture	ND	6	Kigali	87	African buffalo	ND	49.6	Kigali
60	Muriture	ND	4	Kigali	88	African buffalo	ND	47.1	Kigali
61	Muriture	ND	6.8	Kigali	89	African buffalo	ND	47.5	Kigali
62	Muriture	ND	4.9	Kigali	90	African buffalo	ND	48.6	Kigali
63	Muriture	ND	8.2	Kigali	91	African buffalo	42.6	ND	Kigali
64	Muriture	ND	5.6	Kigali	92	African buffalo	44.3	ND	Kigali
65	K'bamba	37	ND	Kigali	93	Royal castle	ND	44.2	Kigali
66	K'bamba	45	ND	Kigali	94	African buffalo	ND	39.7	Kigali
67	K'bamba	36	ND	Kigali	95	Royal castle	ND	39.6	Kigali
68	K'bamba	ND	34	Kigali	96	Rabiant	ND	46.5	Kigali
68	K'bamba	ND	38	Kigali	97	Royal castle	ND	43.2	Kigali
70	K'bamba	ND	30	Kigali	98	Royal Castle	ND	45.2	Kigali
71	Unbranded	ND	27.2	North	99	African buffalo	ND	39.9	Kigali
72	Unbranded	ND	36.9	North	100	African Gin	ND	39.8	Kigali
73	Unbranded	ND	39.6	North	101	K'bamba	ND	50.3	Kigali
74	Unbranded	ND	40.8	North	102	African Gin	ND	31.4	Kigali
75	Unbranded	ND	36.7	North	103	Merry cane	ND	33.2	Kigali
76	K'Bamba	ND	47.9	North	104	African Gin	ND	41.7	Kigali
77	African buffalo	ND	41	Kigali	105	K'bamba	33.7	ND	Kigali
78	K'bamba	ND	43	Kigali	106	K'bamba	ND	36	Kigali
79	Unbranded	ND	12	Kigali	107	K'bamba	ND	32	Kigali
80	African buffalo	40.9	ND	Kigali	108	K'bamba	32	ND	Kigali
81	African buffalo	ND	45	Kigali	109	K'bamba	35	ND	Kigali
82	African buffalo	ND	47.5	Kigali	110	K'bamba	ND	60	Kigali
83	African buffalo	ND	47.4	Kigali	111	K'bamba	ND	32	Kigali
84	African buffalo	ND	49.7	Kigali	112	K'bamba	ND	31.9	Kigali

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Table III. Methanol and ethanol concentrations (% v v^{-1}) in analysed samples (2021-2022) and sample collection details, with red-highlighted values indicating levels exceeding Rwanda Ministry of Health limits and subsequent legal consequences (continuation)

Case No.	Sample Brand	Me-OH(%vv^{1})	Et-OH(%vv^{-1})	Collection Area	Case No.	Sample Brand	Me-OH (%vv^{-1})	Et-OH (%vv^{-1})	Collection Area
113	K'bamba	ND	42.3	Kigali	141	Muriture	ND	6.2	Kigali
114	K'bamba	ND	41.7	Kigali	142	Unbranded	58.3	ND	Kigali
115	K'bamba	ND	34.6	Kigali	143	Merry cane	42.1	ND	Kigali
116	K'bamba	ND	39.8	Kigali	144	Merry cane	37.5	ND	Kigali
117	K'bamba	ND	38.1	Kigali	145	Merry cane	ND	35.7	Kigali
118	Rabiant	ND	41.6	Kigali	146	Muriture	ND	7.5	Kigali
119	Rabiant	ND	40.7	Kigali	147	Muriture	ND	7.8	Kigali
120	Rabiant	ND	38.5	Kigali	148	Unbranded	ND	98.9	Kigali
121	Rabiant	ND	39.6	Kigali	149	African Gin	ND	33.3	Kigali
122	Rabiant	ND	39.1	Kigali	150	African Gin	ND	40.8	Kigali
123	Rabiant	ND	37.7	Kigali	151	Rabiant	ND	37.5	Kigali
124	Rabiant	ND	43.2	Kigali	152	Rabiant	ND	41	Kigali
125	Rabiant	ND	38.1	Kigali	153	Unbranded	ND	49.8	Kigali
126	Unbranded	ND	43.8	Kigali	154	Unbranded	ND	52.6	Kigali
127	African Gin	ND	34	Kigali	155	Unbranded	ND	29.8	West
128	Unbranded	ND	29.8	South	156	Unbranded	ND	45.4	West
129	Unbranded	ND	32.5	South	157	Unbranded	ND	45.9	West
130	Unbranded	ND	29.8	South	158	Unbranded	ND	36.3	West
131	Muriture	ND	7.8	South	159	Unbranded	ND	44	West
132	Unbranded	ND	34.7	South	160	Unbranded	ND	34.9	West
133	Unbranded	ND	35	South	165	Unbranded	ND	40	South
134	Unbranded	ND	23.5	South	166	Unbranded	ND	41.2	South
135	Unbranded	ND	24.1	South	167	Unbranded	ND	96.8	South
136	Muriture	ND	9.1	South	168	African Gin	ND	28.3	South
137	Unbranded	ND	15.1	South	169	Unbranded	ND	13	South
138	Unbranded	ND	40	South	170	Unbranded	ND	11	South
139	Muriture	ND	3.8	South	171	Unbranded	ND	32.5	South
140	Unbranded	ND	39.6	South	172	Unbranded	ND	41.2	West

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Table III. Methanol and ethanol concentrations (% v v^{-1}) in analysed samples (2021-2022) and sample collection details, with red-highlighted values indicating levels exceeding Rwanda Ministry of Health limits and subsequent legal consequences (continuation)

Case No.	Sample Brand	Me-OH(%vv^{1})	Et-OH(%vv^{-1})	Collection Area	Case No.	Sample Brand	Me-OH (%vv^{-1})	Et-OH (%vv^{-1})	Collection Area
173	Unbranded	ND	90.2	West	179	Unbranded	ND	33.7	East
174	Unbranded	ND	33.6	West	180	African gin	ND	39	East
175	Unbranded	ND	16.7	West	181	Muriture	ND	8	East
176	Unbranded	ND	30.9	West	182	Muriture	ND	8.4	East
177	African Gin	ND	43.9	East	183	Unbranded	ND	42.7	East
178	African Gin	ND	43.5	East					

[1]ND: Not Detected. [2]Me-OH: Methanol. [3]Et-OH: Ethanol.

Table IV. Analysing ethanol and methanol levels in samples: A comprehensive breakdown by collection area, brand, and sample count

Collection Area	**Samples per Collection Area**	**Brand or Source**	**Samples per Brand**	**Samples with high Me-OH content (>0.5% vv^{-1}) per brand**	**Samples with high Et-OH content (>45% vv^{-1}) per brand**	**Samples with high Me-OH content (>0.5% vv^{-1}) per area**	**Samples with high Et-OH content (>45% vv^{-1}) per area**	**Samples with high Me-OH content >0.5% vv^{-1} (% of total samples collected)**	**Samples with high Et-OH content >45% vv^{-1} (% of total samples collected)**
City of Kigali	80	Unbranded	6	1	3	12	16	6.5	8.7
		K'bamba	21	6	1				
		Muriture	9	0	0				
		African Buffalo	14	3	8				
		African Gin	6	0	0				
		Rabiant	11	0	1				
		Royal Castle	9	0	3				
		Merry Cane	4	2	0				
Northern Province	61	Unbranded	57	0	9	0	11	0	6.0
		K'bamba	1	0	1				
		Muriture	2	0	0				
		African Buffalo	0	0	0				
		African Gin	1	0	1				
		Rabiant	0	0	0				
		Royal Castle	0	0	0				
		Merry Cane	0	0	0				

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Table IV. Analysing ethanol and methanol levels in samples: A comprehensive breakdown by collection area, brand, and sample count (continuation)

Collection Area	Samples per Collection Area	Brand or Source	Samples per Brand	Samples with high Me-OH content (>0.5% vv^{-1}) per brand	Samples with high Et-OH content (>45% vv^{-1}) per brand	Samples with high Me-OH content (>0.5% vv^{-1}) per area	Samples with high Et-OH content (>45% vv^{-1}) per area	Samples with high Me-OH content >0.5% vv^{-1} (% of total samples collected)	Samples with high Et-OH content >45% vv^{-1} (% of total samples collected)
Southern Province	22	Unbranded	17	0	1	0	1	0	0.5
		K'bamba	0	0	0				
		Muriture	4	0	0				
		African Buffalo	0	0	0				
		African Gin	1	0	0				
		Rabiant	0	0	0				
		Royal Castle	0	0	0				
		Merry Cane	0	0	0				
Western Province	11	Unbranded	11	0	3	0	3	0	1.6
		K'bamba	0	0	0				
		Muriture	0	0	0				
		African Buffalo	0	0	0				
		African Gin	0	0	0				
		Rabiant	0	0	0				
		Royal Castle	0	0	0				
		Merry Cane	0	0	0				
Eastern Province	9	Unbranded	4	0	0	0	0	0	0
		K'bamba	0	0	0				
		Muriture	2	0	0				
		African Buffalo	0	0	0				
		African Gin	3	0	0				
		Rabiant	0	0	0				
		Royal Castle	0	0	0				
		Merry Cane	0	0	0				

Discussion

A total of 183 samples of illicit alcoholic beverages were collected from different regions across the country. The collection comprised 80 samples from the City of Kigali, 61 from the northern province, 22 from the southern province, 11 from the western province, and 9 from the eastern province. These samples were further classified into various brands based on their original labelling, including K'bamba (22 samples), Muriture (17 samples), African Buffalo (14 samples), African Gin (11 samples), Royal Castle (9 samples), Merry Cane (4 samples), and unbranded alcoholic beverages (95 samples).

The study used a Headspace gas chromatography method to analyse methanol and ethanol levels in samples, resulting in strong correlation coefficients (R^2) of 0.997 for methanol and 0.998 for ethanol. The method showed low imprecision, with %RSD values not exceeding 10.1%. The accuracy ranged between 92% and 114%, indicating consistent results across concentration levels. The calibration ranged from 0.01 g% to 1 g% (w v^{-1}), allowing precise quantification. The study adhered to Rwanda's Ministry of Health guidelines, ensuring acceptable levels of methanol and ethanol in alcoholic beverages do not exceed 0.5% and 45% (v v^{-1}), respectively.

In a comprehensive analysis of various provinces and the City of Kigali, we observed remarkable differences in the content of methanol and ethanol in tested samples. In the city of Kigali, which had a sample size of 80 (43.7%), it was found that 15% of these samples, exceeded the permissible limit of 0.5% (v v^{-1}) for methanol. A breakdown of these samples revealed varying percentages from different sources or brands, with 7.5% from K'bamba, 3.75% from African Buffalo, 2.5% from Merry Cane, and 1.25% from unbranded samples. Moreover, 20% of the Kigali samples surpassed the allowed limit of 45% (v v^{-1}) for ethanol, with 10% from African Buffalo, 3.75% from Royal Castle, 3.75% from unbranded sources, 1.25% from K'bamba, and 1.25% from Rabiant.

Turning our attention to the Northern province with 61 samples (33.3%), we observed that all samples adhered to the acceptable limit for methanol levels, indicating a high level of compliance. However, when examining ethanol levels, we found that 18% of the samples (11 out of 61) exceeded the allowable limit. Among these samples, 82% were from unbranded samples, 9% were from K'bamba, and another 9% from African Gin.

In the Southern province, where 22 samples (12%) of total samples were collected, a positive trend was observed, with 100% of the samples meeting the acceptable limit for methanol levels. However, in the case of ethanol, only 4.5% of the samples (1 out of 22), specifically from the African Buffalo brand, exceeded the permitted limit. In the western province, which had 11 (6%) of tested samples, all samples (100%) adhered to the acceptable limit for methanol levels, indicating strong compliance. However, when considering ethanol levels, we found that 27.2% of the samples (3 out of 11), all sourced from unbranded samples, exceeded the allowable limit of ethanol. Lastly, in the eastern province with 9 (4.9%) of tested samples, a perfect compliance rate of 100% was observed for both methanol and ethanol levels, indicating a strong adherence to the acceptable limits for methanol and ethanol.

The study findings also revealed that unbranded alcoholic beverages constituted a significant proportion of the samples collected, accounting for 51.9% out of 183. Meanwhile, K'bamba made up 12%, Muriture 10.3%, African Buffalo 7.6%, African Gin 6%, Rabiant 6%, Royal Castle 4.9%, and Merry Cane 2.1% of the sampled beverages. Notably, the majority of unbranded samples, specifically 48.6% of total collected samples, were collected from provinces outside the city of Kigali, the capital. This suggested that most people in rural regions consume home-brewed and locally-made alcoholic beverages due to their affordability in terms of production and consumption.

Out of 183 samples collected from different parts of Rwanda, including the City of Kigali, in response to suspected cases of methanol poisoning, about 6.6% of the samples (12 out of 183) had methanol levels higher than the permissible limit of 0.5% (v v^{-1}). It is noteworthy that the city of Kigali was the only location for all 12 of these positive samples. The percentage of samples in the same pool that had ethanol levels higher than the allowable limit of 45% (v v^{-1}) was about 16.9% (31 out of 183). Of these 31 samples, about 51.6% came from Kigali, approximately 35.5% from the Northern province, approximately 9.7% from the

Western province, and approximately 3.2% from the Southern province. Significantly, no samples from the Eastern province had ethanol and/or methanol over the allowed limit. Particularly, none of these samples tested positive for both methanol and ethanol simultaneously.

CONCLUSIONS

The study examined 183 samples of illicit alcoholic beverages from various parts in Rwanda, applying a precise Headspace Gas Chromatography method to determine methanol and ethanol levels. The results revealed significant variations in compliance with Rwanda's regulatory limits across different provinces. It is significant to highlight that samples containing ethanol ranged from: 3.8% to 98.9% (v v^{-1}) for ethanol-containing samples and 32% to 58.3% (v v^{-1}) for the samples that had methanol identified.

The City of Kigali exhibited notable non-compliance, with 15% of the samples exceeding the acceptable methanol limit and 20% surpassing the ethanol limit. These issues were attributed to specific brands or sources, including K'bamba, African Buffalo, Merry Cane, Royal Castle, and unbranded alcoholic samples or alcoholic beverages produced locally.

The Northern province showed strong compliance with methanol levels but had 18% of samples exceeding the ethanol limit, primarily from unbranded samples. The Southern province had a perfect compliance rate for methanol but saw a minimal non-compliance rate of 4.5% for ethanol from the African Buffalo brand. The Western province had complete compliance with methanol levels, but 27.7% of samples exceeded the ethanol limit, all from unbranded alcoholic samples. The Eastern province exhibited perfect compliance for both methanol and ethanol.

Overall, approximately 6.6% of the samples had excessive methanol levels, and roughly 16.9% exceeded the ethanol limit. The City of Kigali was the main contributor to non-compliance in both categories. Importantly, no samples tested positive for both methanol and ethanol simultaneously. These findings underscored the importance of monitoring and regulating the alcoholic beverages market in Rwanda to ensure compliance with acceptable methanol and ethanol levels and protect public health.

Conflicts of interest

The authors declare that there is no conflict of interest regarding financial or potential sources of bias such as affiliations, funding sources and financial or management relationships which may constitute a conflict of interest.

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