Research Article | Nepal Journal of Health Sciences
Submitted:30/11/2022
Accepted:20/12/2022
Published:25/12/2022

Determination of Alcoholic Concentration in Four Different Home Brewed Alcoholic Beverages Using Gas Chromatography

Sushma Kaphle,[1] Apurba Acharya,[2] Arbin Shakya,[3] Bijay Aryal[1]

[1]*Department of Clinical Pharmacology, Karnali Academy of Health Sciences, Jumla, Nepal;*
[2]*Department of Forensic Medicine and Toxicology, Karnali Academy of Health Sciences, Jumla, Nepal;*
[3]*Department of Forensic Medicine, Bheri Hospital, Nepalgunj, Banke, Nepal.*

ABSTRACT

Introduction: Alcohol, one of the most commonly consumed beverages across the world is manufactured both industrially as well as locally in Nepal. The proportions of locally brewed alcoholic beverages are higher due to geographical variation as well as presence of a population with varied cultural background. Even though the industrially manufactured alcoholic beverages are quantified, the home brewed beverages usually are produced and consumed without quantification. These alcoholic beverages consist of various components such as ethanol, methanol, iso-propanol, iso-butanol and others, each component having different pharmacological properties.

Objectives: To determine the alcoholic concentration (ethanol, methanol, iso-propanol and iso-butanol) of four different varieties of home brewed alcoholic beverages in Jumla district, Nepal.

Methods: Probability random sampling method was used for sample collection and sampling. The samples were taken to Zest laboratory, Bhaktapur. The laboratory then analysed all four samples to determine the concentration of different alcohols using Gas Chromatography. Unknown concentrations of different alcohol in the sample were measured by injecting standard sample with known concentration.

Results: The concentration of alcoholic components in four different home brewed alcoholic beverage samples were determined using gas chromatographywith ethanol concentrations being 14.209%v/v, 16.323%v/v, 11.473%v/v and 49.217%v/v. Contamination was not detected in any of the samples except for one sample which contaminated iso-butanol (0.063% v/v).

Conclusion: Various alcoholic components are present in home brewed alcoholic beverages at varied concentration levels and quantification of these beverages is highly essential in order to prevent health hazards associated with these components.

Keywords: Alcoholic beverages; alcohol concentration; home brewed alcohol; gas-chromatography; Nepal.

INTRODUCTION

Alcohol is a psychoactive drug and its beverages are one of the most commonly used agents for recreational purpose since ancient times.[1] These beverages consist of various alcoholic components which are ethanol (main component), methanol, iso-propanol, iso-butanol and others. Depending upon their concentration, these components can manifest various effects on human body as they possess certain pharmacological properties.[2] Manufactured at industrial and at household levels in different parts of the world including Nepal, the industrially prepared alcoholic beverages are quantified whereas home-brewed ones are not.

With the altitude of Jumla as high as 4679 meters from the sea levels and temperatures as low as –14°c during the winters,[3] the rate of production and consumption of home brewed alcoholic beverages is significantly higher because of cold temperatures, lesser manufacturing cost and difficult accessibility of industrially manufactured beverages due to inadequate road or airway transportation facilities. So these

Correspondence
Dr. Sushma Kaphle
Email: sushmisharma.sk@gmail.com

Citation
Kaphle S, Acharya A, Shakya A, Aryal B: Determination of Alcoholic Concentration in Four Different Home Brewed Alcoholic Beverages Using Gas Chromatography. Nepal J Health Sci. 2022 Jul-Dec;2(2):56-9.

alcoholic beverages need to be accurately and reliably assessed as they can be hazardous to human health.

Thus, the present study intends to determine the concentration of four different alcoholic components in four different varieties of home brewed alcoholic beverages, namely Chhyang, Local Raksi, Nigar and Local apple cider of Jumla using Gas Chromatography (GC).

METHODS

The study is a cross-sectional study. Home brewed common alcoholic beverages produced in Jumla were included and industrially manufactured alcoholic beverages and beverages produced outside Jumla were excluded. Probability random sampling method was used for sample collection and sampling.

The samples were collected from only municipality, Chandannath. Among the wards of the municipality, lottery method was used to select a ward for collection of samples by random sampling method.

After the ethical clearance from the institutional review committee at Karnali Academy of Health Sciences (Ref no:2078/2079/29), four different types of home brewed alcoholic beverages were collected from the selected ward from four different sites. The amount of each sample collected was 1000ml (1litre), in air-tight bottles, sealed and labeled as sample- 1, sample- 2, sample- 3 and sample- 4 prior to transfer from Jumla. The detail of the labels where ascertained by the principal investigators and were not disclosed to the laboratory. The samples so labeled as sample- 1, sample- 2, sample- 3 and sample- 4 were chhyang, local raksi, nigar and local apple cider respectively. The samples were then transferred to Zest laboratory at Bhaktapur, Nepal.

The laboratory then analysed all four samples to determine the concentration of different alcohols. Unknown concentration of different alcohol in the sample was measured by injecting standard solution with known concentration. The certified reference standard was obtained from Sigma-Aldrich. Gas chromatography was calibrated for standard reference solution of ethanol, methanol, iso-propanol, iso-butanol and distilled water, which was prepared using 1ml of each alcohol (standard reference solution of ethanol, methanol, iso-propanol and iso-butanol) in 50ml volumetric flask and distilled water was added up to the mark. The solution was then filtered through 0.22µ filter.

The sample was prepared by measuring and pouring 5 grams of each test samples in another 50 ml columetric flask and diluted upto mark with distilled water. The solution was filtered by 0.22µ filter. This prepared sample was used for gas chromatographic analysis.

The peak for each sample was obtained twice in chromatogram. The peak value so obtained was then used to calculate the concentration of each sample. The details of the chromatographic system have been stated in table no 1.

Table 1: Chromatographic system.

Parameter	**Value**		
Apparatus	Gas Chromatography (Agilent, made in USA)		
Column	DB-WAX COLUMN (40m x 180µm x 0.3µm.)		
Flow rate	0.6 ml per minute.		
Detector	FID (Flame Ionised Detector)		
Detector temperature	280°Celcius		
Inlet port temperature	250°Celcius		
Injection volume	1µl		
Column Temperature	Ramp rate	Temperature	Hold time
		40°C	3 min
	10˚/min	80°C	2 min
	10˚/min	110°C	5 min

RESULT

The concentration of four different alcoholic components in all the four samples was obtained using the following formula:

(Area of Sample/ Area of Standard) X (Volume of standard / Dilution of Standard) X (Dilution of Solution / Volume of Solution) X (Purity of Standard / 100) X 100%

The alcoholic constituents in all the samples were identified based on the retention time mention in table 2.

Ethanol concentration in sample-1, sample- 2, sample- 3 and sample- 4 were found to be 14.209%v/v, 16.323%v/v, 11.473%v/v and 49.217%v/v respectively. No amount of methanol and iso-propanol were detected in any of the four samples. However, 0.063 %v/v of iso-butanol was detected in sample- 4. The summary of these findings and the main ingredient used to manufacture these beverages locally have been stated in table 2.

In case of sample- 1 and sample- 3, the main ingredient used to manufacture the alcoholic beverage is a grain: namely kodo-millet and rice respectively. But the main ingredient used to manufacture sample- 2 and sample- 4 is rice. However, the difference between the concentrations of the main alcoholic component ethanol in these two samples is significantly high and this difference is owing to the manufacturing process of these alcoholic beverages: local raksi and local apple cider.

DISCUSSION

From its discovery in the 1950s to the present day, gas chromatography has become the gold standard method for quantification of alcoholic beverages throughout the world. [4] A study on the concentration of ethanol in various home brewed alcoholic beverages in Nepal was conducted in 2015 whereby the alcohol content was determined by Pycnometer method.[5] The present study thus becomes the first of its kind in Nepal where quantification of home brewed alcoholic beverages has been done using GC. Another study conducted in Ghana in 2017 whereby methanol and ethanol concentrations in local and foreign alcoholic drinks and food products was quantified using Gas Chromatography and contrary to the present study, the result showed some level of methanol which did not pose any threat to the human body when consumed.[6] Similar to the present study, Headspace GC was used to determine the ethanol content in Kombucha in 2020 in Canada and showed the alcohol level above the limit which can be a potential health risk to children and fetus. [7]

Any alcoholic beverage when consumed in excess amount can lead to toxicity. An alcoholic beverage consisting of methanol, isopropanol, ethylene glycols have been referred to as toxic alcohol.[8] The concentration of methanol less than 5mg/dl is regarded as maximum safe dose. [9]However, higher concentration of ethanol in an alcoholic beverage means lesser toxicity of methanol as ethanol inhibits toxicity of methanol.[10] But this can lead to ethanol toxicity as a consequence of chronic use. Methanol

Table 2: Summary of the concentrations of ethanol, methanol, iso-propanol and iso-butanol in the analyzed alcoholic samples

			Ethanol (%v/v)	Methanol (%v/v)	Iso-propanol (%v/v)	Iso-butanol (%v/v)
Retention time (min)			7.731	7.053	7.584	9.599
Alcohol type	**Main Ingredient**	**Sample Codes**				
Chhyang	(Kodo) Millet	Sample-1	14.209	-	-	-
Local Raksi	Apple	Sample-2	16.323	-	-	-
Nigar	Rice	Sample-3	11.473	-	-	-
Local Apple Cider	Apple	Sample-4	49.217	_	_	0.063

is highly neurotoxic and can induce fatal metabolic acidosis and more commonly blindness due to formic acid accumulation. [11]Iso-propanol is not as toxic as methanol but fatality may be due to inebriant effects such as airway compromise and rarely cardiovascular depression. [8] In case of iso-butanol, its long term exposure leads to slight to moderate skin irritation and severe eye irritation. [8]

Alcohol consumption at higher levels for a prolonged period of time is associated with health hazards from malnutrition to altered hepatic as well as immunological functions. [12]With the consumption of industrially manufactured beverages, the concentration of alcoholic components can be easily obtained and thus the toxicity associated with it can be ascertained. But this is not true with consumption of home brewed alcoholic beverages in which the concentration of various alcoholic components are not provided and different beverage manufactured from same ingredients can also produce toxicity due to varied concentration as shown in the present study. Hence, it becomes highly essential to quantify any alcoholic beverage so that the levels of toxicity can be assessed based upon its composition and concentration.

CONCLUSION

Various alcoholic components present in alcoholic beverages can be harmful to human health based upon their concentration and constituent of many home brewed alcoholic beverages are not quantified most of the time. As such it becomes highly essential that the commonly used home brewed alcoholic beverages be quantified upon on a larger scale in order to prevent health hazards and fatality as discussed above, especially in places in a country like Nepal where its production and consumption is significantly higher than the industrially manufactured alcoholic beverages. Further, the study also emphases on the importance of having GC facilities at different regional levels as in its absence it become highly difficult to carry samples from one geographical area to another.

ACKNOWLEDGEMENT

We would like to thank the Zest laboratories and research center, Bhaktapur, Nepal for their immense support.

Conflict of Interest: None

NJHS

REFERENCES

1. Botelho G, Anjos O, Estevinho LM, Caldeira I. Methanol in grape derived, fruit and honey spirits: A critical review on source, quality control, and legal limits. Processes. 2020 Dec;8(12):1609. [Full Text / DOI]
2. Rajbhandari R, Acharya N, Agrawal J. Methanol Poisoning: Our Experience with Recent Outbreak. Nepal J of Neuroscience. 2008;5:75-7. [Full Text / DOI]
3. District Profile. Rural Access Programme [Internet]. Archive.rapnepal.com.2022 [cited 16 February, 2023]. Available from: http://archive.rapnepal.com/district/district-profile-12.
4. Lacorn M, Hektor T. Determination of ethanol in kombucha, juices, and alcohol-free beer by EnzytecTM Liquid Ethanol: single-laboratory validation, First Action 2017.07. Journal of AOAC International. 2019 Jan 1;102(1):316-8. [PMID: 29618403/ DOI]
5. Thapa N, Aryal KK, Paudel M, Puri R, Thapa P, Shrestha S, Stray-Pedersen B. Nepalese Homebrewed Alcoholic Beverages: Types, Ingredients, and Ethanol Concentration from a Nation Wide Survey. Journal of Nepal Health Research Council. 2015 Jan 1;13(29):59-65. [PMID: 26411715 / Full Text]
6. Tulashie SK, Appiah AP, Torku GD, Darko AY, Wiredu A. Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Gakenkey, Fantekenkey and Hausa koko) in Ghana. International Journal of food contamination. 2017 Dec;4(1):1-5. [Full Text]
7. Chan M, Sy H, Finley J, Robertson J, Brown PN. Determination of ethanol content in Kombucha using headspace gas chromatography with mass spectrometry detection: single-laboratory validation. Journal of AOAC International. 2021 Jan;104(1):122-8. [PMID: 33099632 / Full Text /DOI]
8. Patocka J, Kuca K. Toxic alcohols: aliphatic saturated alcohols. Mil. Med. Sci. 2012;508:142-63. [Full Text /DOI]
9. Zamani N, Rafizadeh A, Hassanian-Moghaddam H, Akhavan-Tavakoli A, Ghorbani-Samin M, Akhgari M, Shariati S. Evaluation of methanol content of illegal beverages using GC and an easier modified Chromotropic acid method; a cross sectional study. Substance abuse treatment, prevention, and policy. 2019 Dec;14:1-7.[Full Text / DOI]
10. Md C, Elena T, Ibolya F, Erzsébet F. A survey on the methanol content of home distilled alcoholic beverages in Transylvania (Romania). ActaMarisiensis-SeriaMedica. 2013 Aug;59(4):206-8. [Full Text / DOI]
11. Shapira B, Schaefer E, Poperno A, Hess Z, Rosca P, Berkovitz R. The methanol content of illicit alcoholic beverages seized in a low socio-economic area of Tel-Aviv: Public health impact and policy implications. Journal of Public Health. 2019 Feb;27(1):37-42. [Full Text / DOI]
12. Ahmed FE. Toxicological effects of ethanol on human health. Critical reviews in toxicology. 1995 Jan 1;25(4):347-67. [PMID: 7576156/ DOI]