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Investigation of Brewing Water Hardness and Alkalinity, and Ethanol Content in Nepalese Home-Brewed Alcoholic Beverages in a Locale of Pokhara Countryside

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Abstract

The current study determined brewing water hardness, alkalinity, and ethanol content in non-distilled and distilled home-brewed alcoholic beverage (HBAB) samples available in the local households of Pokhara, Nepal countryside. The HBAB samples selected for the ethanol content determination were (i) non-distilled home-brewed alcoholic beverages *bhate jand* & *chhyang* prepared by fermentation of a mashed mixture of glutinous steamed rice and starter culture media of marcha (yeasts) and *tongba* prepared by fermentation of a mashed mixture of steamed finger millet and marcha (yeasts) starter, and (ii) distilled HBAB *viz. local raksi* prepared by fermentation of a mashed mixture of millet grains and yeasts followed by the subsequent distillation in different lots in household distillery plants. The study revealed desirable hardness but excess alkalinity factors in the brewing water samples. The non-distilled HBABs were found to have less than half the ethanol content in distilled HBABs. Among the non-distilled HBABs, steamed rice fermented *chhyang* had higher ethanol concentration than glutinous rice fermented *bhate jand* and finger millet fermented *tongba* beverages. The standard guidelines for brewing water hardness and alkalinity and standardization of ethanol content are essentially required for branding and commercialization and restricted consumption of the Nepalese HBABs. This study provides useful data for national economic and health plans for these purposes.

Keywords: Home brewed alcoholic beverages; Ethanol content; Brewing water alkalinity and hardness; Fermentation; Non-distilled and distilled alcoholic beverages

1. Introduction

Hard water refers to water with a higher concentration of bicarbonate, sulfate, and chloride of calcium or magnesium. Hardness corresponds to a property of water because it does not lather easily with soap. It forms scale in boilers, cooling towers, kettles, and water heaters (Harris, 2010; Pal et al., 2018). The hardness of water is measured in terms of the concentration of dissolved polyvalent cations, commonly calcium and magnesium ions, as the contribution to the hardness of manganese, iron(II), and strontium often appears lower than a minimum threshold concentration (Pal et al., 2018). Total hardness is also attributed to the overall concentration of alkaline earth ions (Eßlinger et al., 2009; Kadlec, 2002), which is the sum of carbonate and non-carbonate hardness. The counter ions for carbonate hardness are bicarbonate ions that are converted into insoluble carbonate on boiling (Kadlec, 2002; Briggs et al., 1981).

Still, the counter ions of non-carbonate hardness are chloride, sulfate, and nitrate, and these ions remain unchanged in boiling (Eßlinger et al., 2009; Briggs et al., 1981). The hardness of water is expressed as calcium carbonate equivalent concentration in mg per liter of water (ppm). Water with this concentration below 43 ppm is soft, 43-150 ppm is slightly hard, 150-300 ppm is moderately hard, and 300-450 ppm is hard. Water above 450 ppm is taken as very hard water (Pal et al., 2018). Hard water has positively impacted brewing technology, specifically on amylase activity in the wort mashing. The brewing wort prepared from hard water has higher original gravity (OG) values that correspond to solid contents in the brewing wort before the commencement of alcoholic fermentation (Puncocharova et al., 2018). Hard water-brewed beers have been found to have more vitamin B2 contents, possibly due to the formation of stable complexes of bivalent alkaline earth ions with these vitamin materials (Puncocharova et al., 2018). Both the caustic alkalinity caused by carbonate and hydroxide ions and the bicarbonate alkalinity caused by bicarbonate ions refers to the capacity of water to neutralize an acid (Gray, 2017; Patton, 1991). The increase in pH due to the presence of these ions is due to their combination with H^+ ions to give neutral species. Estimating alkalinity regarding calcium carbonate equivalent in mg/L-1 involves determining the amount of acid required to lower the pH to 4.2, at which alkali is completely used up (Dhoke, 2023). Thus, alkalinity prevents pH fluctuations, usually comprising the buffering system of carbonic acid, carbonate, and bicarbonate in equilibrium (Palmer & Kaminski, 2013). The alkalinity of wort and alcoholic beverages increases with increased brewing water alkalinity (Palmer, 2016). Hence, the alkalinity of brewing water is essentially maintained below 100 mg/L-1 calcium carbonate equivalents (Palmer & Kaminski, 2013) to prevent the undesirable flavor profile associated with excessive alkalinity of water as a major ingredient (Anderson et al., 2019). Consumption of alcoholic beverages with ethanol as a major constituent has raised economic, social, and health problems throughout the world (Room et al., 2003; World Health Organization, 2011). Despite the serious health issues, consumption of home-brewed alcoholic beverages (HBABs) that comprise both the non-distilled beverages prepared from rice (Tamang, 2006) and millet cereals (Thapa et al., 2004) and the distilled liquors commonly prepared from cereals fruits and sugar (Thapa et al., 2015) has been practiced in Nepal since the ancient times (Thapa et al., 2015). Ethanol content in alcoholic beverages ranges from 7-21% (v/v) in wine, 20-50% (v/v) in liqueur and 3-6% (v/v) in beer (Anonymous, 1992). Ethanol content is a quality index and flavor indicator for beverages (Wang et al., 2003). In a nationwide cross-sectional study, the average ethanol concentration in Nepal's commonly available HBAB was higher than the ethanol content in beer and wine markets but less than in branded liqueurs (Thapa et al., 2004). The current study comprises the assessment of brewing water hardness and alkalinity, and ethanol content in non-distilled and distilled HBABs in a research locale of Nepal, Pokhara countryside, to generate the relevant data associated with variations in fermentation and distillation techniques, ingredients composition, brewing water source, hardness, and alkalinity.

2. Materials and Methods

2.1.Collection of samples

Six samples of non-distilled HBAB *viz.* two *bhate jand* samples, two *chhyang* samples, and two *tongba* samples with the respective brewing water samples, and six samples of distilled HBAB *viz.* millet fermented *local raksi* with the respective brewing water samples were aseptically collected in sterile and airtight polypropylene bottles from the household distilleries in the countryside of Pokhara in Nepal. The bottles were stored at an ambient temperature of 25°C in the Laboratory of Chemistry, Pashchimanchal Campus, Pokhara. The experiments were accomplished a month after the arrival of samples in the laboratory.

2.2.Materials

For brewing water hardness determination, the complexometric titration: 0.01 M solution of ethylenediaminetetraacetate (EDTA) disodium di-hydrate salt, $C_{10}H_{14}N_2Na_2O_8.2H_2O$, Sigma Aldrich, chelator reagent, minimum percentage purity 98.5, molecular weight (M_w) 372.24; Eriochrome black T (EBT) indicator, $C_{20}H_{12}N_3NaO_7S$, Sigma Aldrich, percentage purity 99.5, M_w 461.38; Basic buffer solution (solution of an equimolar mixture of ammonia and ammonium chloride). For brewing water alkalinity measurement: Phenolphthalein (3,3-Bis(4-hydroxyphenyl)-1(3H)-isobenzofuranone 3,3-Bis(4-hydroxyphenyl)-2-benzofuran-1(3H)-one) indicator $C_{20}H_{14}O_4$, Merck, solution prepared as 1% m/v in 95% solution of ethanol with percentage purity 99.8%, M_w 318.32; Methyl orange (4-[4-(Dimethylamino)phenylazo]benzenesulfonic acid sodium salt) indicator $C_{14}H_{14}N_3NaO_3S$, Merck, solution prepared by adding 0.1 g of methyl orange in 80 mL of distilled water and 20 mL of 95% ethanol; 0.1 N sulphuric acid solution (prepared by dilution of sulphuric acid concentrate, H_2SO_4, M_w 98.08, Merck; For determination of ethanol content in HBAB samples, distillation followed by specific gravity (SG) measurement using pycnometer (50 mL capacity/ SG hydrometer, short range (0.96 – 1.00): Distilled water, Laboratory Reagent (LR) grade, prepared by steam distillation, H_2O, M_w 18.02. All chemicals and reagents were of analytical grade and used without further purification.

2.3.Calculations

2.3.1. Brewing water hardness

1000 mL of 1 M EDTA solution ≡ 40 g of Ca^{2+} ions ≡ 100 g of $CaCO_3$
x mL of 0.01 M EDTA solution ≡ $\frac{x}{1000}$ g of $CaCO_3$
Hardness corresponding to 50 mL of brewing water = $\frac{x}{1000}$ g of $CaCO_3$
Hardness corresponding to 1000 mL of brewing water = $\frac{20\,x}{1000}$ g of $CaCO_3$ = 20x mg of $CaCO_3$. Hence brewing water hardness is 20x ppm. (Pal et al., 2018) [2, 23]

2.3.2. Brewing water alkalinities

The alkalinity measurement involved endpoint determination in the titration of a brewing water sample against a standard acid solution in the presence of phenolphthalein in the first phase and methyl orange in the second phase. Alkalinity was calculated with the equation:

$$\text{Alkalinity (ppm as CaCO3)} = \frac{\text{Volume of acid consumed (mL) X normality of acid (N) X 50 X 1000}}{\text{Volume of brewing water sample (mL)}}$$

The first phase of the phenolphthalein endpoint showed complete neutralization of OH^- and half neutralization of carbonate ions *viz.* ½ CO_3^{2-}. Further, the second phase or methyl orange endpoint showed complete neutralization of OH-, CO32-, and HCO_3^-. So, after the determination of phenolphthalein alkalinity (P) and methyl orange alkalinity (T), the alkalinities attributed to different ions were calculated with the help of interrelations between P and T, as shown in Table 1 (Maiti, 2004; Manahan, 2010; Patton, 1991).

Table 1: Alkalinities due to different ions and their interrelations

Case no	Titration results	Hydroxide ions	Carbonate ions	Bicarbonate ions

		alkalinity	alkalinity	alkalinity
Case 1	P = 0	Absent	Absent	T
Case 2	P = T	T	Absent	Absent
Case 3	P = ½ T	Absent	2P	Absent
Case 4	P > ½ T	2P – T	2 (T-P)	Absent
Case 5	P < ½ T	Absent	2P	T – 2P

2.3.3. Ethanol content in HBABs: Pycnometer method of determination of specific gravity (S.G.)

The ethanol content in HBABs was determined using the pycnometer method to determine the specific gravity of the distillate (Anonymous, 1992; AOAC Official Method of Analysis, 1990). The S.G. values of distilled HBAB and the distillate of non-distilled HBAB were calculated with the equation: $S.G. = \frac{W1-W}{W2-W}$ and the corresponding alcohol percentages (v/v) were found in the 'Annexure I: Relation between S.G. & Alcohol Percentage' table in the Manual of Methods for Analysis of Alcoholic Beverages, Food Safety and Standards Authority of India (Ministry of Health and Family Welfare) FDA Bhawan, Kotla Road, New Delhi (Food Safety and Standards Authority of India, 2019), and Indian Standard, Table of Alcoholometry (Pyknometer method) First revision (2005), Bureau of Indian Standards, Manak Bhawan, 9 Bahadur Shahzafar Marg, New Delhi, India, accessed at https://law.resource.org/pub/in/bis/S11/is.3506.1989.pdf (Bureau of Indian Standards, 2005).

2.4. Experimental

2.4.1. Measurement of brewing water hardness

The measurement of the overall hardness of brewing water involved complexometric titrimetric addition of 0.01M EDTA solution in a mixture of 50 mL of the brewing water, 10 mL of basic buffer solution and two drops of EBT indicator in a titration flask till the wine-red color of the alkaline earth metal-EBT solution turned into blue color of EBT in free state at a pH maintained at 8-10. The process involved the formation of a more stable alkaline earth metal-EDTA chelate by substituting EBT from an alkaline earth metal-EBT complex in the titer (Pal et al., 2018; Joshi et al., 2023).

2.4.2. Measurement of brewing water alkalinity

The brewing water alkalinity in terms of phenolphthalein alkalinity (P) and methyl orange alkalinity (T) was measured with minor modifications in the method given by Dhoke et al. (2023). A brewing water sample (25 mL) with 2 drops of phenolphthalein taken in a conical flask was titrated against 0.1 N sulphuric acid to get P until the faint pink color was discharged. Next, 2 drops of methyl orange indicator were added, and the titer was further titrated against 0.1 N sulphuric acid to measure T at the point of conversion of the yellow color of the solution into pink.

2.4.3. Measurement of ethanol content in HBABs: Pycnometer method of determination of specific gravity (S.G.)

The ethanol content in HBABs was measured by distilling the beverages, measuring the S. G. of the distillate, and determining the ethanol concentration using the reference of comparison annex: specific gravity vs. ethanol percentage. This was carried out by following the procedure duly explained in Manual of Methods for Analysis of Alcoholic Beverages, Food Safety and Standards Authority of India (Ministry of Health and

Family Welfare) FDA Bhawan, Kotla Road, New Delhi-110002, India (Food Safety and Standards Authority of India, 2019) and also with reference to Indian Standard, Table of Alcoholometry (Pyknometer method) First revision (2005), Bureau of Indian Standards, Manak Bhawan, 9 Bahadur Shahzafar Marg, New Delhi, India (Bureau of Indian Standards, 2005).

3. Results and Discussion

The S.G. of the distillate and the corresponding ethanol contents in the non-distilled HBAB *viz. bhate jand* samples (J1, J2), *chhyang* samples (C1, C2) and *tongba* samples (T1, T2), and distilled HBAB *viz. local raksi* samples (R1, R2, R3, R4, R5, R6) are shown in table 2. The hardness of the respective brewing water samples (BWJ1, BWJ2, BWC1, BWC2, BWT1, BWT2 for non-distilled and BWR1, BWR2, BWR3, BWR4, BWR5, and BWR6 for distilled HBAB) with the titrimetric data are presented in table 3. The alkalinities of these brewing water samples (BWJ1, BWJ2, BWC1, BWC2, BWT1, BWT2 for non-distilled and BWR1, BWR2, BWR3, BWR4, BWR5, and BWR6 for distilled HBAB) with the titrimetric data are presented in Table 4.

Table 2: The specific gravity (S.G.) of the distillates and the corresponding ethanol contents in the non-distilled and distilled HBABs

Type of HBAB	Sample	S.G.	Ethanol (v/v %)
Non–distilled	J1	0.9923	5.4
	J2	0.9921	5.5
	C1	0.9913	6.2
	C2	0.9913	6.2
	T1	0.9920	5.6
	T2	0.9920	5.6
Distilled	R1	0.9821	13.8
	R2	0.9816	14.2
	R3	0.9821	13.8
	R4	0.9814	14.4
	R5	0.9814	14.4
	R6	0.9816	14.2

Table 3: The hardness of brewing water samples for non-distilled and distilled HBABs with the titrimetric data

Type of HBAB	Sample	For total hardness		For permanent hardness	
		Concurrent burette reading (mL)	Hardness ($CaCO_3$ eqiv., ppm)	Concurrent burette reading (mL)	Hardness ($CaCO_3$ eqiv., ppm)
	BWJ1	15.0	300	6.8	136

Non–distilled *viz. bhate jand*, chhyang & tongba	BWJ2	15.0	300	6.8	136
	BWC1	14.8	296	6.5	130
	BWC2	14.8	296	7.0	140
	BWT1	15.0	300	6.8	136
	BWT2	14.8	296	6.5	130
Distilled *viz. local raksi*	BWR1	15.0	300	7.2	144
	BWR2	15.0	300	7.0	140
	BWR3	14.7	294	6.8	136
	BWR4	14.7	294	6.8	136
	BWR5	15.0	300	7.0	140
	BWR6	15.0	300	7.0	140

Table 4: The alkalinities of brewing water samples for non-distilled and distilled HBABs with the titrimetric data (P: phenolphthalein and T: methyl orange endpoint)

Type of HBAB	Brewing water sample	P (mL)	T (mL)	Total alkalinity (ppm)	Carbonate ions alkalinity (ppm)	Bicarbonate ions alkalinity (ppm)
Non–distilled	BWJ1	0.15	0.50	100	60	40
	BWJ2	0.15	0.50	100	60	40
	BWC1	0.10	0.65	130	40	90
	BWC2	0.10	0.50	100	40	60
	BWT1	0.15	0.65	130	60	70
	BWT2	0.10	0.65	130	40	90
Distilled *viz. local raksi*	BWR1	0.15	0.60	120	60	60
	BWR2	0.10	0.60	120	40	80
	BWR3	0.15	0.50	100	60	40
	BWR4	0.10	0.65	130	40	90
	BWR5	0.15	0.50	100	60	40
	BWR6	0.15	0.50	100	60	40

The non-distilled HBABs traditionally prepared in Nepal are *bhate jand* and *chhyang* (white rice beers) from steamed rice fermented with yeast mould mixture and *tongba* from steamed finger millet fermented with yeast *khesung* culture starter. The distilled liqueur HBABs in households are more commonly obtained from millet grains fermented with *marcha* as the yeast culture starter. The current study showed more than double the percentage (v/v %) of ethanol content in distilled HBAB *viz. local raksi* (13.8-14.4%) than in non-distilled alcoholic beverages viz. *bhate jand* (5.4-5.5%), *chhyang* (6.2%) and *tongba* (5.6%). The HBAB *chhyang* had the highest ethanol content among the non-distilled alcoholic beverages. The ethanol content in these non-distilled alcoholic beverages was less than the reported 7-12.9% wine concentration and higher than the average US and European beer concentration of 4.5% (Dufour, 1999). The Pokhara-based Nepalese distilled HBABs were found to have higher ethanol concentration than the home-brewed alcoholic beverages in Uganda (6-11%), and these non-distilled Nepalese HBABs were stronger than the home-brewed beverages of Ghana (2-3%), Egypt (3.8-4.2%), and Ethiopia (2-4%). However, these Nepalese-distilled HBABs were weaker than Indian-distilled liqueur at 22-45% and Kenyan change spirit at 34% (World Health Organization, 2004). Ethanol concentration in the jand samples was close to the reported ethanol concentration of 5.9% in rice in India (Tamang & Thapa, 2006), and the ethanol concentration in chhyang samples was less than the reported concentration of 8.2% in similar home-brewed beverages in India (Kanwar et al., 2011). The difference in ethanol concentration in similar beverages may have been attributed to maturation time, the lot (first, second, or third) of the distillate of fermented grains, the mixed ingredients (fruit, sugar), cereal type, fermentation performance, and procedure of preparation (Thapa et al., 2015). The non-distilled HBABs in the Himalayan region of Nepal, Bhutan, and India are mild strength, calorific, and inexpensive beverages (Tamang, 2006). The rice with its sweetened taste is of better quality (Thapa & Tamang, 2004). The household preparation of non-distilled HBABs involves cooking starch grains or sugar source, mixing it with yeast (marcha) as a culture starter, and a few days o*f* alcoholic fermentation in an earthen pot (Kanwar et al., 2011; Tamang et al., 1996; Tsuyoshi et al., 2005). The preparation of distilled HBAB involves the additional steps of collection of distillates in first, second and third lots (Thapa et al., 2015). The traditional production and consumption of HBABs in Nepal (Sapkota et al., 2009; Jhingan et al., 2003; Dhital, 2001; WHO STEPS Surveillance, 2008), inclusively by a substantially high women population (WHO STEPS Surveillance, 2008), have brought about massive alcohol use disorder (AUD) rates, internalized stigma, suicidality, depression Rathod, S. D., Luitel, N. P., & Jordans, M. J. D. (2018), and cancer (Shrestha et al., 2022).

Brewing water quality affects the enzymatic activity of yeasts in wort and the taste and quality of alcoholic beverages. The flavor, clarity, and stability of alcoholic beverages are affected by brewing water hardness (Puncocharova et al., 2018) and alkalinity (Dhoke, 2023). The total hardness of the brewing water samples (294-300 ppm) was found within the moderately hard water range at 150-300 ppm (Pal et al., 2018) and in a suitable range of calcium ions concentration at 50-150 ppm (The power of pH., n.d.). The alkalinity data corresponded to case 5 in Table 1 *viz.* $P < ½ T$ showing P is less than T-P of titration. $P = OH^- + ½ CO_3^{-2}$, but since $OH^- = 0$, $P = ½ CO_3^{-2}$ or $2P = CO_3^{-2}$. It showed that 2P was the volume of acid consumed for neutralizing only CO_3^{-2} in the brewing water samples. $T = OH^- + CO_3^{-2} + HCO_3^-$, but since $OH^- = 0$, $T = CO_3^{-2} + HCO_3^-$ or $HCO_3^- = T - 2P$. It showed that T-2P was the volume of acid consumed for neutralizing only HCO_3^- in the brewing water samples. The results showed that the hydroxide ions were nil, which clearly indicates the reaction of predominantly existing bicarbonate ions with hydroxide ions to give carbonate ions and water. The total alkalinity of the brewing water samples (100-130 ppm) was above the upper acceptable alkalinity of 100 ppm. It showed the unsuitability of these water samples for brewing the HBABs of better flavor and quality. Furthermore, a suitable chemical or biological treatment of the brewing water towards moderate lowering of its alkalinity is recommended on the basis of this study. The current type of on-site research study may be limited by factors such as seasonal variations in hardness and alkalinity of the brewing water, pollution effects, fermentation mold mixture quality and ratio, fermentation process, and distillation practices.

4. Conclusions

Brewing water hardness and alkalinity-associated pH factors must be assessed and maintained at an appropriate range for producing better-quality alcoholic beverages. The current investigation showed that the brewing water used to prepare Pokhara-based Nepalese HBABs had a suitable hardness to add flavor, clarity, and quality to beverages. Still, the total alkalinity was beyond the maximum, showing the necessity of reducing the brewing water pH and alkalinity. The study revealed that ethanol content (v/v%) in non-distilled HBABs was less than half the ethanol content in distilled HBABs. The ethanol content among the non-distilled HBABs was found to be higher in *chhyang* than in *bhate jand* and *tongba* beverages. The ethanol content might have been affected by factors such as types of cereal and ingredients, ratio of ingredients, fermentation procedure, yeast enzymatic activity, and maturation time. The data generated from this investigation are useful for economic and health policymakers, academia, and researchers to standardize and regulate Nepalese HBAB production for branding, commercializing, and restricting alcohol-related unhealthy practices.

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