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Semi-quantitative analysis of nucleic acids on agarose gels with choice of beverages as electrophoretic buffer

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Abstract
The pH of beverages is known to be low, and has therefore been involved in the increasing acidity incidence. In this study, to report on a rapid and cost-effective approach for nucleic acid characterization using different running buffers for agarose gel electrophoresis. Commercial DNA is used in electrophoretic analysis using a variety of beverages by optimizing its pH value. The results suggest that Royal Stag has little effect on nucleic acid research, while Peter Scot shows high concentration and sensitivity in nucleic acid characterization. Royal Stag drink has revealed that concentration, time, and voltage have little effect on nucleic acid research, although Peter Scot has reported high concentration and sensitivity in nucleic acid characterization. We conclude that this approach is simple, easy, and cost-effective compared to using TAE or TBE as running buffers in electrophoresis of the agarose gel.

Keywords: Antiquity, peter scot, royal stag, bagpiper, TAE, electrophoresis

Introduction
Agarose gel electrophoresis is a widely used technique for separating DNA fragments based on their size. The gel matrix is made of agarose, a linear polysaccharide derived from seaweed, which has a high gel strength that allows for the handling of low percentage gels suitable for separating large DNA fragments [1]. The size of the pores formed by the agarose bundles in the gel matrix is determined by molecular sieving, meaning that smaller DNA fragments can pass through the pores more easily than larger fragments. The performance of agarose gel electrophoresis is influenced by the properties of the electrophoresis buffer, such as pH, ion strength, and composition [2]. The choice of buffer can affect the migration of DNA fragments, and researchers often experiment with different buffer systems to optimize their results. Positive polarity buffer systems typically work over the entire pH spectrum, whereas negative net-charge nucleic acids are delayed on the gel surface in buffer systems with negative polarity [3-5]. New methods have been developed to improve the resolution of agarose gel electrophoresis [6]. Multiple gradient-slab gel electrophoresis involves embedding several agarose gels with different concentrations within a single gel slab, allowing for more precise separation of DNA fragments. However, difficulties in using organic solvents with low buffer capacity can require buffer adjustments or the use of a buffer recirculation system [7]. Overall, agarose gel electrophoresis remains a widely used and versatile technique in biological sciences research [8]. With continued experimentation and development of new methods, it is likely to remain an important tool in molecular biology [9].

Materials and Methods
Determination of pH in alcoholic beverages
Various drinks, including Bagpiper, Peter Scot, Sprite, Royal Stag, and Antiquity, were chosen from local market of Vijayawada, Andhra Pradesh, India, Ten millilitres of each sample were tested for initial pH and adjusted to typically a pH of 8.3 using either 1M NaOH or HCl.

Using milliQ water or double-distilled water, the buffer was diluted to a concentration of 100 times, then stored at 40°C until needed [10].

Agarose gel electrophoresis

Genei's small mini-subcell (10 x 12 cm) was used for electrophoresis of the agarose gel and used beverages as a running buffer to classify nucleic acids. One microgram of commercial DNA (Salmon sperm DNA, SRL) is used as a loading sample along with a tracking dye. Beverages as buffers were measured using a DC-300 power supply (Genei Apparatus) with length, voltage, current and power of 300 ml. Fifteen microliters of samples were prepared, and a CCD camera (UVI-Tech, Germany) stained gel with ethidium bromide for UV photography [11].

Quantification of gel band

ImageJ 1.38 (http:/rsb.info.nih.gov/nih-image/) is used as a method to measure the agarose gel band strength for the analysis of gel photos. Gel is coated with ethidium bromide and tested for UV-transilluminator band validation. Gels was put in a gel doc (UVI-Tech, Germany) and photographed. ImageJ is used for measuring the density profile, peak height, and peak intensity or band volume of the expected molecular weight [12].

Results and Discussion

Determination of pH in beverages: Beverages are the most acidic according to American Dental Association. In fact, their acid content is in the same range as vinegar. Initial pH of all selected beverages was determined using pH meter (EuTech, Germany) after the pH sensor is calibrated as listed in table 1. Experiment was carried out for three times to obtain more accurate results.

Table 1: pH in units of several beverages

Name of the beverage	pH in units
Bagpiper	2.79
Peter Scot	2.58
Royal Challenge	4.58
Royal Stag	4.89
Antiquity	2.44
Distilled water (Negative control)	7
TAE Buffer (Positive control)	8.3

Agarose gel electrophoresis

Commercial DNA of 15ul was loaded in each well, and electrophoresis was performed at 50 volts for one hour at 50 volts. Each band contains a large quantity of DNA fragments of the same size, all of which have travelled to the same position as a group. Gel is stained with a DNA-binding dye and placed under UV light, allowing us to see the DNA present at various positions along the length of the gel. Water at neutral pH as negative control of the reaction is used as running buffer, while TAE buffer as positive control. No bands were observed with water as choice and high-density bands were observed with TAE buffer as shown in figure 1.

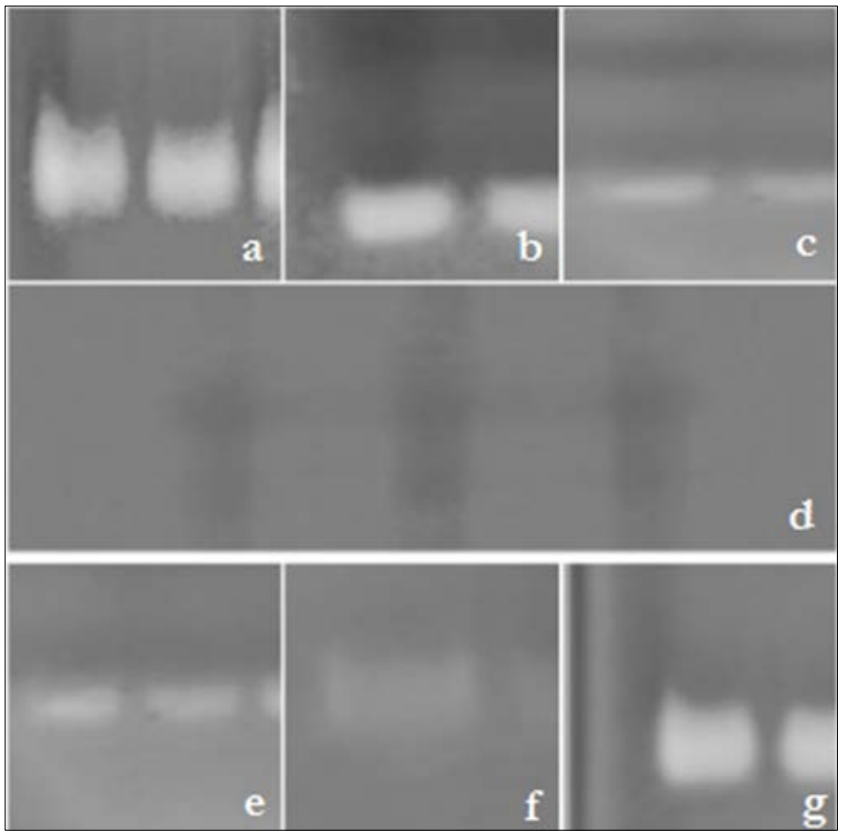

Fig 1: Bands of DNA observed under UV-transilluminator and photographed using gel-doc. a-g: Bagpiper as buffer; Peter Scot as buffer; Royal Challenge as buffer; water as buffer; Royal Stag as buffer; Antiquity as buffer and TAE as buffer.

Quantification of gel band: The analysis of the ECL image was performed using the public domain ImageJ program (developed at the National Institutes of Health and available at http:/rsb.info.nih.gov / ij/), using the "Measure" function. The results of the analysis are a value for each band that is proportional to the Integrated Density Value (IDV) of the band. All photographs have been handled in a way that prevents the loss of information as shown in table 2.

Table 2: Quantification of band intensity using ImageJ

S. No	Label	Area	X	Y	Major	Minor	IntDen
1	Bagpiper	1548	81.500	27	145.561	13.541	266249
2	Peter Scot	3674	89.500	35	188.439	24.824	623466
3	Royal Challenge	1572	79.500	28	147.818	13.541	273148
4	Redbull	1449	84.500	25.500	181.669	10.155	227615
5	Antiquity	1056	93	29	198.595	6.770	168869
6	Water	380	30	33	42.878	11.284	64145
7	TAE	2700	95	19.500	203.108	16.926	389126.000

Conclusion

This study suggests that the method described is not intended to replace TAE buffer, but rather to serve as a preliminary indicator of possible trends in the choice of beverages for nucleic acid characterization using agarose gel electrophoresis. The study found that Peter Scot had high precision and sensitivity compared to other beverages tested, including Bagpiper, Royal Challenge, Antiquity, and Royal Stag. However, the results may vary if more beverages were included in the study. The study also suggests that further studies could be carried out to analyze RNA as genetic material using gel electrophoresis. However, it is important to note that the method described in this study is limited in scope and may not be appropriate for all nucleic acid characterization experiments. The choice of running buffer and other experimental conditions should be carefully considered based on the specific research question and sample being analyzed. Nonetheless, the study provides some preliminary insights into the potential use of alternative running buffers for agarose gel electrophoresis in nucleic acid characterization.

Authors' contributions

All the authors have contributed equally.

Conflict of interest

The authors declare that there are no conflicts of interest exist among them regarding the publication of this paper.

References

1. Huang Q, Baum L, Fu WL. Simple and practical staining of DNA with GelRed in agarose gel electrophoresis. Clinical Laboratory Journal For Clinical Laboratories And Laboratories Related. 2010 Jan 1;56(3):149.
2. Vemuri PK, Talluri B, Sharma A, Akkala G, Bodiga VL. Isolation and Characterization of a Lactose-Binding Lectin from Ocimum sanctum. Journal of Applied Pharmaceutical Science. 2015;5(10):113-7.
3. Calladine CR, Collis CM, Drew HR, Mott MR. A study of electrophoretic mobility of DNA in agarose and polyacrylamide gels. Journal of molecular biology. 1991 Oct 5;221(3):981-1005.
4. Vemuri PK, Talluri B, Panangipalli G, Kadiyala SK, Veeravalli S, Bodiga VL. Purification and Identification of 20kDa protein from Parthenium hysterophorus. International Journal of Pharmacy and Pharmacognosy Research. 2016;8:827-30.
5. Tiselius A. Electrophoresis of serum globulin. I. Biochemical Journal. 1937 Feb;31(2):313. 6.
6. Rodbard D, Chrambach A. Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. Analytical biochemistry. 1971;40(1):95-134.
7. Fischer SG, Lerman LS. Length-independent separation of DNA restriction fragments in two-dimensional gel electrophoresis. Cell. 1979;16(1):191-200.
8. Theodoridis GA, Papadoyannis IN. Novel advanced approaches in sample preparation and analyte detection for bioanalysis. Current Pharmaceutical Analysis. 2006;2(4):385-404.
9. Kumar VP, Prasanthi S, Reddy AC, Raj ND, Anudeep L. Characterization studies of thermostable alkaline phosphatase from various plant seeds. Journal of Applied Biosciences. 2010;36:2403-8.
10. Kumar Vemuri P, Veeravalli S. Expression, purification and characterization of human recombinant galectin 3 in Pichia pastoris. Iranian Journal of Biotechnology. 2014;12(2):3-8.
11. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature methods. 2012;9(7):671.
12. Heidebrecht F, Heidebrecht A, Schulz I, Behrens SE, Bader A. Improved semiquantitative Western blot technique with increased quantification range. Journal of immunological methods. 2009;345(1-2):40-8.