



Soft beverages as an alternative to TAE buffer in agarose gel electrophoresis

Niranjan Babu Akula¹, Manav Vanga², Suryanarayana Veeravilli³

^{1,2} Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India

³ Department of Humanities and Basic Sciences, Aditya Engineering College, Surampalem, Kakinada, East Godavari District, Andhra Pradesh, India

Abstract

Background: The pH of carbonated drinks is known to be low and have, therefore, been implicated in the increasing incidence of acidity.

Aim: Here we report on an efficient approach for rapid analysis of nucleic acid characterization by varying a cost-effective running buffers for agarose gel electrophoresis.

Material and Methods: Commercial DNA is used in electrophoretic analysis using various soft beverages by optimizing its pH value and gel bands were quantified using Image.

Results: Red bull energy drink revealed that concentration, time and voltage do not have any effect in analysis of nucleic acids, while fizz revealed high concentration and sensitivity in characterization of nucleic acids.

Conclusion: This approach was fast, easy and cost-effective than use of TAE or TBE as running buffers in agarose gel electrophoresis.

Keywords: Thumsup, Fizz, Red bull, Pepsi, TAE, Electrophoresis

Introduction

Since the adoption of agarose gel in the 1970s for the separation of DNA, it has proven to be one of the most useful and versatile technique in biological sciences research [1]. Agarose's high gel strength allows for the handling of low percentage gels for the separation of large DNA fragments [2]. Molecular sieving is determined by the size of pores generated by the bundles of agarose in the gel matrix [3]. Properties of electrophoresis buffers such as pH, ionic strength, and composition affect performance [4]. Choice of novel buffers seem more likely to be widely adopted when researchers do not have to commit to purchasing expensive machines [5]. Buffer systems of a positive polarity function normally over the entire pH range, whereas nucleic acids with negative net charge are retarded at the gel surface in buffer systems with negative polarity [6]. New methods were also developed by embedding several agarose gels as multiple gradient-slab gel electrophoresis each of a different agarose concentration, within a single gel slab [7]. Difficulties also hinders the use of organic solvents in the running buffer has an extremely low buffering capacity, necessitating buffer changes or the use of a buffer recirculation system [8]. In comparison to TAE and TBE, soft beverages buffers provide higher voltage and current stability, lower working concentration and lower voltage, resulting in less heat generation.

Material and methods

Determination of pH in soft beverages

Various soft beverages like Pepsi, Fizz, Sprite, Red bull and Thumsup were selected based on the convenience and availability at KL University campus, Andhra Pradesh, India. Ten ml of each sample were checked for initial pH and adjusted with either 1M NaOH or HCl to attain usually

to pH 8.3. The buffer was diluted to 100 times using double distilled water or milli Q water and store at 4°C until further use [9].

Agarose gel electrophoresis

Wide mini-sub cell from Genei (10 X 12 cm) was used for agarose gel electrophoresis and carried out using soft beverages as running buffer for characterization of nucleic acids [10]. Commercial DNA (Salmon sperm DNA, SRL) of 1µg in concentration is used as loading sample along with tracking dye. Soft beverages as buffers were tested with 300ml volume, voltage, current, and power were recorded with an DC-300 power supply (Genei Apparatus). Fifteen microliters of sample were loaded, gel was stained with ethidium bromide for UV photography by a CCD camera (UVI-Tech, Germany).

Quantification of gel band

For analysis of the gel images Image J 1.38 (Windows version of NIHImage, <http://rsb.info.nih.gov/nih-image/>), is used as tool for quantification of band intensity on the agarose gel. Gel is stained with ethidium bromide and analysed on UV-trans illuminator for the confirmation of bands. Gels were placed in gel doc (UVI-Tech, Germany) and photographed. Image J is used for density profile measurement, peaks height as well as peak intensity or volume of the band of the expected molecular weight [11].

Results and discussion

Determination of pH in soft beverages

Soft drinks are the most acidic beverages according to Real Water Health. In fact, their acid content is in the same range as vinegar. Initial pH of all selected beverages was determined using pH meter (Eu Tech, 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
Pepsi	2.58
Fizz	5
Sprite	3.26
Red bull	2.79
Thumsup	2.44
Distilled water (Negative control)	7
TAE Buffer (Positive control)	8.3

Agarose gel electrophoresis

Commercial DNA of 15ul in each well was loaded and carried out the electrophoresis for one hour at 50 volts. Each band contains a large quantity of DNA fragments of the same size that have all travelled as a group to the same position. Gel is stained with a DNA-binding dye and placed under UV light, allowing us to see the DNA present at different locations 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 [Figure1].

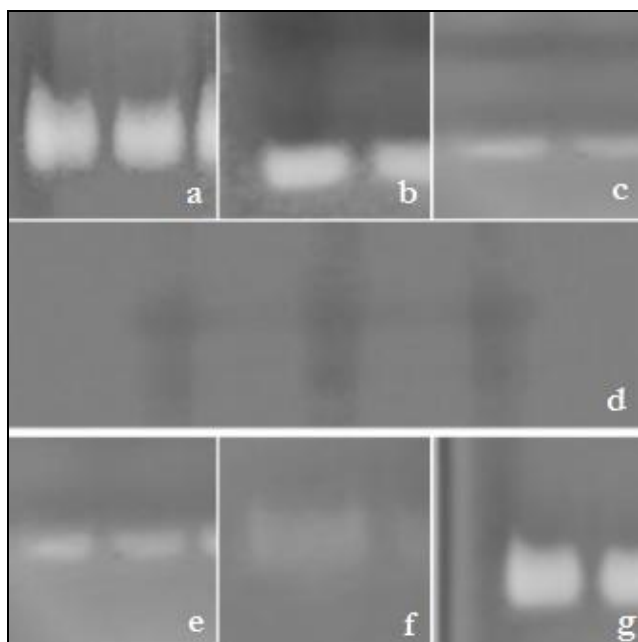


Fig 1: Bands of DNA observed under UV-trans illuminator and photographed using gel-doc. a-g: pepsi as buffer; fizz as buffer; sprite as buffer; water as buffer; redbull as buffer; thumsup as buffer and TAE as buffer.

Quantification of gel band

Analysis of ECL image was performed using the public domain Image J program (developed at the National Institutes of Health and available at <http://rsb.info.nih.gov/ij/>), using the “measure” functions. Results of the analysis is a value for each band which is proportional to the Integrated Density Value (IDV) of the band. All images were treated in a way that avoids loss of information [12].

Table 2: Quantification of band intensity using ImageJ

S. No	Label	Area	X	Y	Major	Minor	Int Den
1	Pepsi	1548	81.500	27	145.561	13.541	266249
2	Fizz	3674	89.500	35	188.439	24.824	623466
3	Sprite	1572	79.500	28	147.818	13.541	273148
4	Redbull	1449	84.500	25.500	181.669	10.155	227615
5	Thumsup	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

Based on the study, fizz was observed with high precision and sensitivity and also observed good results among other beverages of pepsi, sprite, mountain dew and red bull. However, the present results might be varied if more beverages were considered for the study. Further studies can be carried out for analysis of the other beverages with gel electrophoresis.

Conflict of interest

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

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