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Effect of Sugars on Apre Gene Abundance in Fermented Locust Bean Seeds

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ABSTRACT

Locust bean seeds are traditionally used in preparation of stews, sauces, soups and more in most parts of Africa, therefore cultivation is essential even in environmental stress conditions. This research aimed at evaluating the effect of different concentrations of sugars on the Alkaline Protease E (APRE) gene abundance on fermented Locust bean seeds. The locust bean seed were made to undergo fermentation aided by Saccharomyces cerevisiae and Lactobacillus sp in varying concentrations (0.1g, 0.2g and 0.4g) of the Maltose, Glucose, Lactose and Sucrose in different containers and kept at room temperature. 5% gel electrophoresis, Polymerase Chain Reaction (PCR) thermal profiles, sample curtails, and primer sequences for APRE gene were employed. Results of locust bean seed fermentation with various sugar concentrations revealed complex changes in protein profiles, indicating a shift in metabolic pathways. It showed a decrease in the abundance of APRE gene with increase in concentrations of Maltose during locust bean fermentation. The highest abundance (Above 80,000) was recorded at 0.1g. Glucose also showed highest abundance of APRE (above 80000) at 0.1g concentration of the sugar. Similarly, the abundance of APRE gene at the concentrations of lactose at 0.1g was above 80000 but a decrease at concentration of 0.4g during locust bean fermentation. For Sucrose, the highest abundance was close to 80,000 was recorded at 0.4g but least abundance (slightly above 40000). Although all the sugar concentrations produced high abundance of APRE gene. Generally, glucose and maltose produced the highest amount of the APRE gene than Sucrose and Lactose at lower concentrations which makes them economically viable. The study concludes that all the sugars stimulated protein expression or enzyme activity but vary in their effects on genes abundance. This gene finds application in the production of ammonia, enzymes, nitrogen fixation, and protein hydrolysis and in other industrial and agricultural processes to boost food security.

1. INTRODUCTION

The Locust bean (*Parkia biglobosa*) is an important legume in West Africa, with high nutritional value. Fermented increases digestibility, nutrient bioavailability, and flavor profiles (Agboola *et al.*, 2010).

Esenwaha and Ikenebomeh (2010) reported that sugar contents particularly glucose and sucrose level in locust bean seeds were generally low. Furthermore, the fermentation of locust bean led to a diminution of between about 69-99% in glucose and sucrose contents correspondingly. However, Olatoye *et al*. (2019) documented that different species of locust beans are likely to produce unpredictable levels of glucose and sucrose. Many factors such as soil, sunlight, rain fall, as well as processing methods like drying, roasting and the likes can influence the abundance of theses sugars in the seeds.

Fundamental to Locust bean fermentation is the α-amylase-producing regulator (APRE) gene, which regulates the assembly of α-amylase which breaks down maltose from composite carbohydrates.

Locust bean seed enhance the welfare of communities in West Africa and elsewhere (Odibo *et al.*, 2017).

APRE gene also recognized as subtilisin, is an important protease enzyme obtained from the bacterium *Bacillus subtilis* has a vital part in several industrial applications, showcasing distinctive catalytic activities (Takata *et al.,* 2018). APRE's impact covers pharmaceutical, food, textiles, detergents, biofuels and other industries (Takata *et al.*, 2018).

APRE's molecular structure, belonging to the serine protease family, includes multiple domains and a catalytic triad (serine, histidine, and aspartate residues). This compact and globular structure ensures stability and functionality under diverse conditions, making APRE gene reliable for industrial use (Baranyi and Győrvári, 2018).

APRE's enzymatic activity involves the cleavage of peptide bonds in proteins, facilitated by its catalytic triad. The enzyme exhibits substrate specificity, contributing to its versatility in various industrial applications (Takata *et al*., 2018).

APRE plays a crucial role in protein hydrolysis, breaking down proteins into peptides and amino acids. This controlled cleavage of peptide bonds enhances the efficiency and specificity of protein hydrolysis in applications such as biofuel production (Takata *et al.,* 2018). APRE gene restricts proteolysis degradation to ensure protein constancy. In addition they assist retreating the action of proteases which in turn help to sustain protein homeostasis. They provide support against cellular stress to defend the cell from destruction. Furthermore, they inhibit proteolysis breakdown and degeneration which eventually helps to maintain food value and safety thus making them find application in food, pharmaceutical biotechnology industries and more.

Various factors contribute in determining the abundance of APRE gene expression in Locust beans. Some include species of Locust beans, temperature, pH, and oxygen level, moisture content during fermentation, microbial profile, and nature of pre-treatments such as heating, pounding, soaking and environmental factors such as soil climate among others. Generally, the abundance of APRE genes is documented to be specific in tissues with seeds than other organs (Waadte *et al*., 2022; Devi *et al*., 2023). High level of sugar content in fruits is linked to the interface between sugars production and sugar transport gene.

This investigation examines the influence of additives such as glucose, maltose, sucrose and lactose, on APRE gene abundance in Locust beans. This unties the complex relationships between these additives and APRE gene abundance thereby demystifying innovative approaches to handle α-amylase assembly and optimize Locust bean fermentation for boosted nutritional value, food security as well as encouraging economic growth, and

2. Materials and Methods

This study was conducted in the Molecular laboratory and New Biology Laboratory in the College of Biological Sciences, Joseph Sarwuan Tarka University, located in Makurdi, the capital of Benue State, Nigeria.

2.1 Sample collection

Ten (10g) of Locust bean seed samples were collected from Wurukum Markets Makurdi

and stored at -20 °C till the time of processing

2.2 Sample preparation

2.2.1Fermentation of Locust bean seeds

The seeds were soaked in water for 5 minutes, boiled for 6 hours, de-hulled and washed. The washed bean seeds were again boiled for 90 minutes after adding potassium bicarbonate. The de-hulled beans were fermented in a fermenter at controlled temperatures, pressures, pH and relative humidity with *S. ceresiviae* and *Lactobacillus* sp as culture organisms.

Varying concentrations (0.1g, 0.2g and 0.4 g) of Maltose, Lactose, Sucrose and Glucose were added to the fermenting locust beans in different glass fermenters and kept at room temperature.

2.3 DNA extraction

This was performed according to modified protocol described by Sambrook and Russell (2001), Ausubel *et al*.(2015) and Zhang and Chen (2019). In this experimental procedure, 200 mg of locust bean was measured and poured into a 1.5 ml Eppendorf tube. To this, 100 µl of Phosphate Buffer Saline with a pH of 8 was added, followed by the supplementation of 150 µl of a lysozyme solution. The subsequent blend was subjected to incubation period at 37°C for 2 hours. Afterward, 150 µl of SDS solution was added, and thoroughly amalgamated. The combination was incubated at room temperature for 5 minutes, followed by extra 2-minute incubation on ice. Then 20-minute incubation at 65°C was performed and succeeded by additional 2-minute period on ice. To this, 150 µl of 3 M sodium acetate was added, and the solution was thoroughly vortexed. The set up was then centrifuged at 6,000 x g for 10 minutes to obtain the resultant supernatant.

2.4 Experimental Design Structure

2.4.1 5% Gel Electrophoresis

The modified protocol by Sambrook and Russell (2001), Ausubel *et al*.(2015) and Lee and Lee (2019) was employed. A 0.75g of agarose gel was dissolved in 50ml of TBE buffer. 3ul of ethidium bromide, at a concentration of 1:10,000, was poured into the molten gel. The resulting gel was cautiously dispensed and left to set for 15 minutes. Using two gel combs, a whole of 16 wells were produced for sample loading. Gel loading was expedited by adding 1ul of gel loading dye to 5ul of the sample, and this combination was then loaded into the designated well. Furthermore, a separate well was kept for loading 5ul of a 100bp DNA ladder. The gel was subjected to a 35-minute run at a voltage of 100v. Post-run, the gel was observed in a UV transilluminator to picture the detached DNA portions.

Other preparations are the Lysozyme Solution, composed of 150 mM Tris-HCl, 100 mM EDTA, and Lysozyme at a concentration of 15 mg/ml, formulated by combining 3.75 ml of 1M Tris-HCl, 12.5 ml of 0.2 M EDTA, and 375 mg of Lysozyme. The volume was then adjusted to 25 ml using nuclease-free water.

Consequently, a 3 M Sodium Acetate solution was made with a overall volume of 50 ml. This was realized by liquefying 12,304.5 mg of Sodium acetate in 30 ml of MilliQ-Water, fine-tuning the pH to 5.2, and finishing the volume to 50 ml with nuclease-free water.

The preparation of a 0.1 M Phosphate Buffer Saline, comprising of 0.02 M NaH_2PO_4, 0.08 M NaH_2PO_4, and 9% NaCl, was undertaken for a total volume of 50 ml. This involved merging 5 ml of 1 M NaH_2PO_4, 20 ml of 0.2 M NaH_2PO_4, and 4,500 mg of NaCl, regulating the pH to 7.4, and making up the volume to 50 ml with nuclease-free water.

Finally, an SDS Solution was made by blending 1 ml of 1 M NaCl, 5 ml of Tris-HCl, and 1 g of SDS (10%). The solution was meticulously blended, and any consequential foam was left to settle down before usage in the experimental processes. The prepared solutions played a central role in enabling the accuracy and consistency of the conducted experiments.

Table i: PCR thermal Profile

Operation	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5min	1
Denaturation	95	20sec	
Annealing	53	60sec	
Extension	72	40s	25-30
Final Extension	72	5min	1

Table ii: PCR Curtail for each Sample

Component	Volume (ul)
Master Mix	4
Forward Primer (10uM)	0.5
Reverse Primer (10um)	0.5
DNA Template	7
BSA	2
dH_2O	6

Table iii: Primer Sequence

Primer	Primer Sequence	Annealing Temperature
APREF	TATGTTAACGCAGCCACAGC	51.78
APRER	GCCATCACATCAAGCTCAGA	51.78

2.5 Data analysis

Image J was employed for the Annotation and analysis of the gel image

3. Results and Discussions

The results are represented on the figures shown. The three concentrations on the bar charts coincide with the three bands on gel electrophoresis in all the figures presented.

Figure 1 is the result of gel electrophoresis indicating that band B in maltose was the biggest.

In this study the abundance of APRE genes decreases with increases concentration of Maltose. The highest abundance was recorded at 0.1g of maltose. The lowest abundance was recorded at 0.4g of Maltose.

Figure 2 shows a decrease in the abundance of APRE genes with increase in concentrations of maltose during locust bean fermentation. The highest abundance (Above 80,000) was recorded at 0.1g. The link between various sugars and abundance of APRE genes decreased with increase in concentration of maltose. This supports findings of Flores-Santos *et al*. (2020) who reported that increase in concentration of maltose decreased abundance of gene responsible for microbial fermentation. Industrially, very minimal quantity of maltose is needed for optimum production of the gene which is economically viable.

The gel electrophoresis (Figure 3) showing the effect of glucose on APRE gene abundance in locust beans also revealed three distinct bands corresponding to the three different glucose concentrations (0.1g, 0.2g and 0.4g). The band intensity appears strongest and weakest in the sample with the lowest concentration (0.1g) and highest concentration (0.4g) respectively.

Figure 4 showed highest abundance of APRE genes at the least concentrations of glucose (0.1g) during locust bean fermentation. There was a decrease at 0.2g and a slight increase at 0.4g. The highest abundance (Above 80,000) was recorded at 0.1g.

Similarly, an increase concentration in glucose also produced a decrease in abundance of the APRE gene. This could be related with feedback inhibition mechanism which prompts increased sugar levels that provides excess nutrients for the microbes involved in the fermentation to restrict further production.

This refutes the prospect of a unswerving, comparative link between nutrient accessibility and gene expression. These outcomes support observation in other plant species where precise genes show highest expression at lesser nutrients or stress levels (Aremu *et. al.,* 2015). Locust beans might possess glucose sensing mechanisms that activate APRE gene expression at low glucose levels to facilitate proficient application of inadequate resources. Perhaps, these mechanisms become saturated at higher glucose levels (Gibson, 2004; Gang *et al*., 2018 and Fareen *et al*., 2019). The non-linear response witnessed in this work could be due to plant hormones such as auxins which network with glucose and effect APRE gene expression.

The gel electrophoresis (Figure 5) showing the effect of Lactose on AprE gene abundance in locust beans similarly showed three distinct bands corresponding to the three different Locust concentrations (0.1g, 0.2g and 0.4g) (Figure 6). The band intensity (A and B) appears strongest on sample with the lowest concentration (0.1g and 0.2g) and weak (Band C) on samples with highest concentration (0.4g) .

For the lactose, the abundance of APRE gene was constant at lactose level of 0.1g and 0.2g but increased at 0.4g showing general increase with increase in lactose level. This may be linked to the effects of the microbial load during fermentation. *Lactobacillus* sp is most likely to increase in lactose level for its optimum metabolic activity which may in turn influence the production level of the gene in question. Generally speaking, microbes have different affinity for different sugars. *Lactobacillus* sp has high affinity for lactose than *S. cervisiae* and *Bacillus* sp. These all to some level degrade lactose but with variations which in turn may influence the abundance of the APRE genes during fermentation. However, the influence of lactose appears to be contrasting.

The gel electrophorosis(Figure 7) showed that bands B and C were the bigest in sucrose. The bands may be attributed to the induction of specific enzymes crucial for the fermentation process, leading to elevated protein levels or enhanced enzyme activity, including the expression of the APRE gene. This conforms with the study of Feng *et al.* (2018) who reported that increase in band intensity during fermentation indicate stimulatory effects of a catalyst.

Figure 8 represents the least abundance of the genes was recorded at 0.1g (lowest concentration) of sucrose. This implies that sucrose affected the rate of locust beans fermentation by influencing the availability and uptake of APRE genes. Similar result was reported by Feng *et al.* (2022) who documented that sucrose is the preferred sugar for microbial fermentation because it has higher affinity and a faster uptake rate. Flores-Santos *et al.* (2020) reported that it is generally accepted that sucrose in fermentation products such as locus beans produced through extracellular hydrolysis of sugar, mediated by APRE genes and periplasmic invertase.

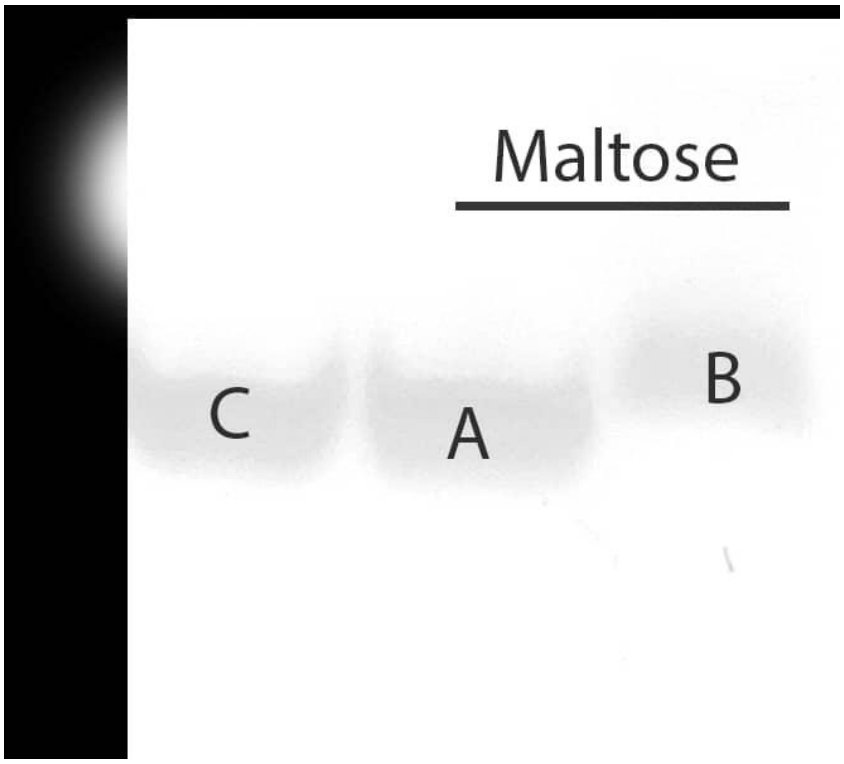

Figure 1: Gel electrophoresis showing effect of varying maltose concentration on APRE Gene abundance in fermented locust beans .

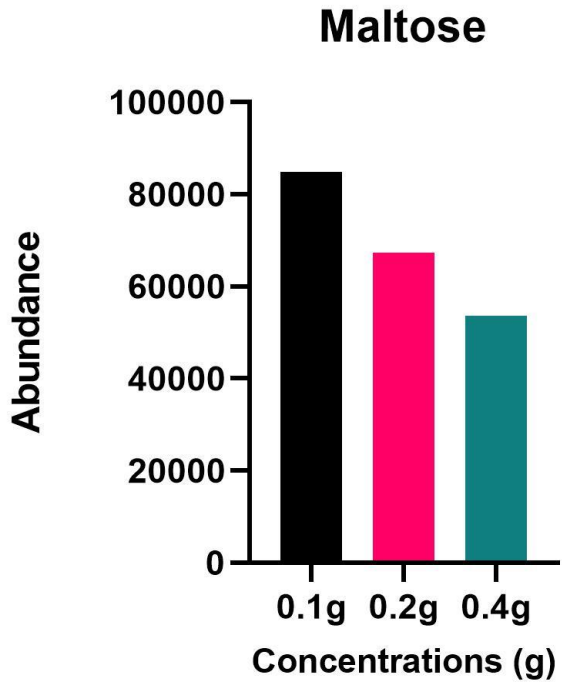

Figure 2: Effect of varying maltose concentration on APRE Gene abundance in fermented locust beans.

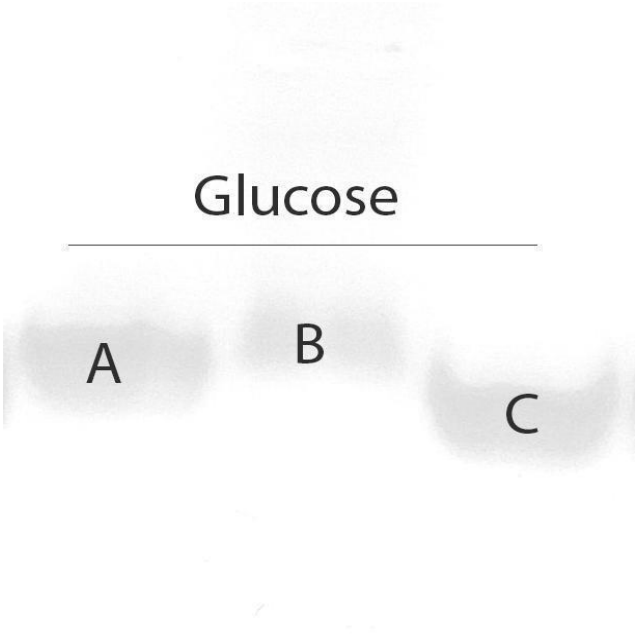

Figure 3: Gel Electrophoresis showing effects of Glucose on APRE gene abundance in fermented locust beans

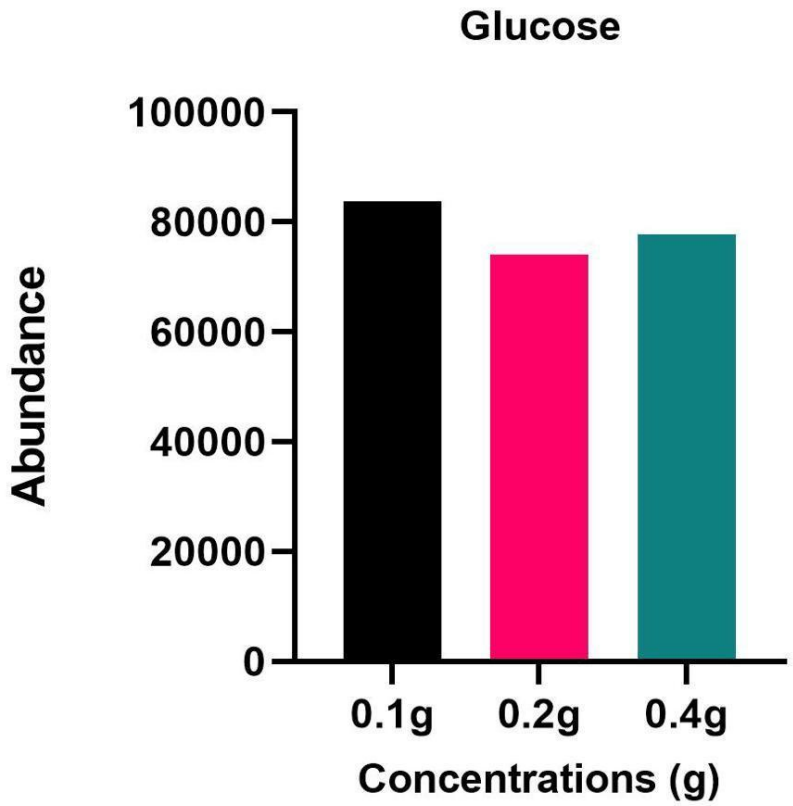

Figure 4: Effect of varying glucose concentration on APRE Gene abundance in fermented locust beans.

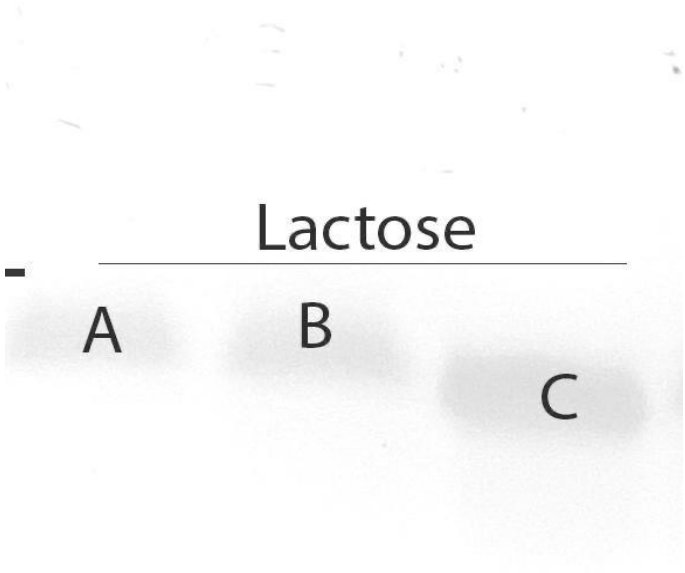

Figure 5: Gel Electrophoresis showing effects of Glucose on APRE gene abundance in fermented locust beans

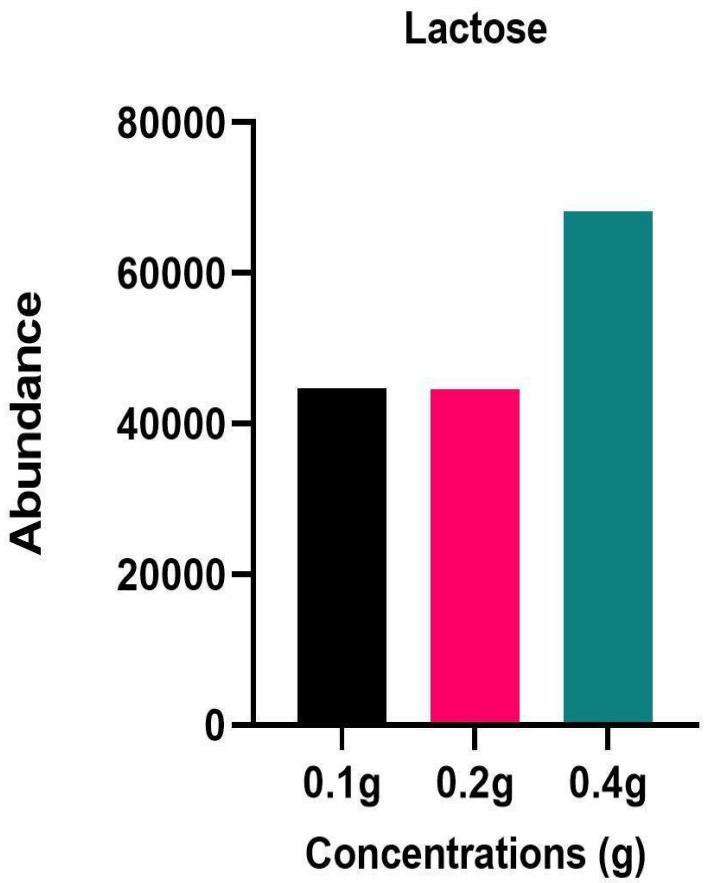

Figure 6: Effect varrying lactose concentration on APRE Gene abundance in fermented locust beans

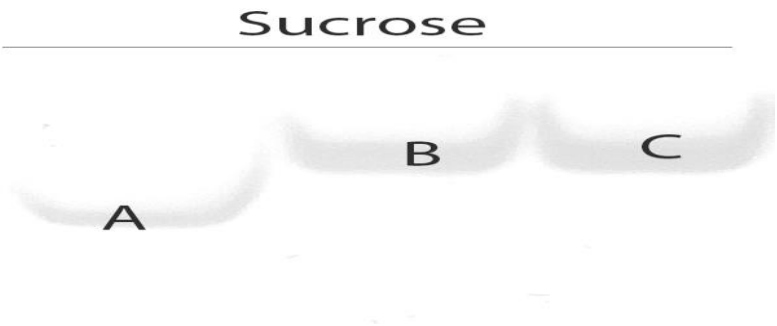

Figure 7: Gel Electrophoresis showing effects of Sucrose on APRE gene abundance in fermented locust beans

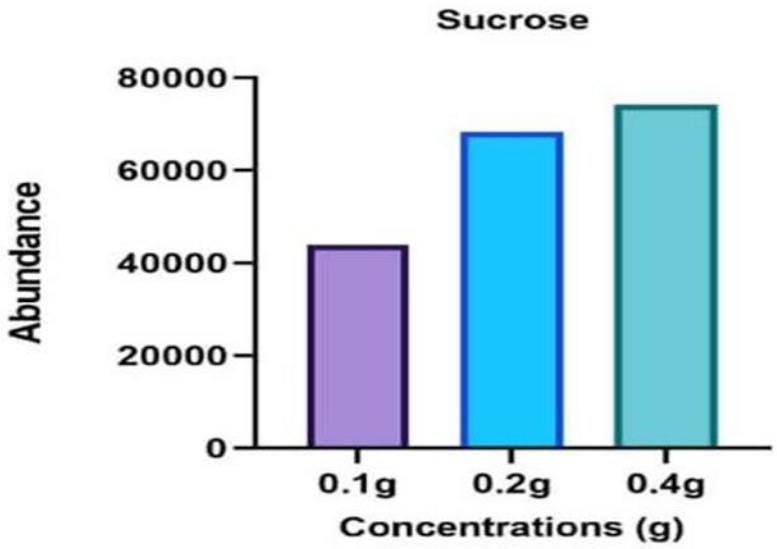

Figure 8: Effect varrying sucrose concentration on APRE Gene abundance in locust beans fermentation

4. Conclusion

Our gel electrophoresis results reveal that sucrose, maltose, glucose and lactose, at certain concentrations, stimulate protein expression or enzyme activity in Locust bean fermentation.

However, their effects on abundance differ. Glucose and maltose showed decrease in abundance of AprE gene with increase in the concentration of the sugars while the opposite was the case for sucrose and lactose during the fermentation of the locust bean seeds. Generally, glucose and maltose produced highest amount of the AprE gene than sucrose and lactose showing their efficiency in low availability or stress conditions making them very economically viable.

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Author contributions

Mabitine D.M, initiated the ideas and supervised the study; Akuete, S.V, Alechenu, S.E, Tyolaha, S, Cletus,G.E conducted the laboratory work and wrote the first draft of the manuscript. Entire authors went through the draft of the write up; all writers acknowledged the text.

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