Proximate Composition of Acid-Treated Wheat Bran Substrate used for Glutamic Acid Production

Hamza Magaji [1*], Aliyu Shuaibu[1], Eberemu Nkiru Charity[2] and Umar Ismail[1]

[1]Department of IJMB Science, Nuhu Bamalli Polytechnic, Zaria, Kaduna, Nigeria
[2]Department of Biological Sciences, Federal University Dutsin-Ma, Katsina, Nigeria
* Corresponding Author:-hmmakarfi@yahoo.com; GSM: 08036649988

Abstract

Wheat bran is one of the highly utilized residues with perceptible amount of carbohydrates and protein which are useful for microbial utilization to produced value-added products.Several different lignocellulosic residues hold remarkablepotential for bioconversion into a valuable industrial product such as glutamic acid, and thereby presenting dual advantage of sustainable resource supply and environmental quality. The study aimed to determine proximate composition of the acid-treated wheat bran used for glutamic acid production by Enterobacter sp. (BUK_BTE 17 MW597320). Two (20) kg of fresh wheat bran (substrate for bacterial growth) was collected from the wheat milling station and the sample was packaged into a polythene bag, labeled appropriately, and transported to the Department of Microbiology, BUK for proximate analysis according the method of AOAC 2005. The results show that the proximate composition analysis of the powdered acid treated-wheat bran sample revealed the Moisture content of 4.27±0.090%, Ash content of 16.43±0.087%, Crude fiber 14.57±0.095%, Crude fat 4.02±0.029%, Crude protein 2.69±0.093%, and Carbohydrate content of 58.03±0.051%, respectively. In conclusion, the use of locally available and nutrient-rich raw material (wheat bran) as a substrate for glutamic acid production is possible and makes the process economical and in turn reduces the environmental pollution by biotransformation to commercially useful bio-product. Different agricultural residues such as potato peels, sugar cane bagasse, and cereal husk should also be tried for their potential to serve as substrates for glutamic acid production.

Keywords: Proximate, Wheat bran, Glutamic acid, *Enterobacter,* and TLC.

INTRODUCTION

In most developing nations such as Nigeria, hundred thousand tons of agricultural residues are produced annually. These residues are often employed as substrates for the generation of various value-added products such as glutamic acid. Wheat bran is one of the highly utilized residues which have a perceptible amount of carbohydrates (Tallur*iet al*., 2019). Wheat bran consists of starchy polysaccharides (10-20%), non-starchy polysaccharides (40-60%), and (10-20%) protein which also makes it important for microbial utilization to produced value-added products (Biswas *et al*., 2019). Wheat bran, a by-product of the dry milling of Triticumaestivum or Triticum aestivum L (Scientific name of wheat. into flour, is among the main agro-industrial by-products utilized in animal feeding. It consists of the outer layers (cuticle, pericarp, and seed coat) combined with small amounts of the starchy endosperm of the wheat kernel. The composition of wheat bran contains different amounts of soluble sugars such as glucose, arabinose, galactose, xylose, etc. These soluble sugars are useful for the initiation of cell growth and replication of microbes (El-Shishtawy *et al*., 2014). However, wheat bran has appreciable amounts of protein and low lignin content as compared to other substrates. However,

wheat bran is preferred as the better substrate for the production of microbial enzymes such as glutamic acid (El-Shishtawy *et al*., 2014). It can be used as the cheapest source of energy for fermentation (Khan *et al*., 2006; Talluri *et al*., 2019). Pre-treatment of the wheat bran increases the surface area and supply of cellulose which may be hydrolyzed to glucose by microorganisms (Shafaghat *et al*., 2010). The utilization of wheat bran as a low-cost carbon source for the production of glutamic acid makes the method economical and successively reduces the environmental land pollution by bioconversion to commercially useful bio-product (Bishir *et al*., 2016).

Proximate analysis is set of methods used to obtain information about the nutritional value of food/feed. It is a chemical scheme of food analysis designed to measure six basic components; moisture content, total ash, ether extract (fat), crude protein, and Nitrogen free extracts (carbohydrates), and thus provide the nutritional value of the feed (AOAC, 2005).
Glutamic acid is among the most important non-essential amino acid that is widely used in foods as a flavor enhancer (Bishir *et al*., 2016). Glutamic acid has been reported to act as a neurotransmitter (Nakayama, 2021). Amino acids are very important biological components commercially used as additives in food, feed supplements, infusion compounds, therapeutic agents, and precursors for peptides synthesis or agriculture-based chemicals (Shyamkumar *et al*., 2014). Glutamic acid not only acts as the precursor for other amino acids and biological molecules (glutathione and adrenalin) etc but plays a significant role in cellular processes such as intracellular signaling, nitrogen metabolism, energy production, and cell wall synthesis. Its highest concentrations are present in the brain and muscles and it is needed on a daily basis for survival (Fahimitabar *et al*., 2021).

Enterobacter sp. is a gram-negative rod with flagella and facultative anaerobic growth, catalase-positive, and oxidase-negative. *Enterobacter sp* requires biotin for cell growth. Biotin is important for glutamic acid synthesis. It had been thought that the cell wall permeability increased when biotin was depleted in the medium. Similarly, the addition of detergent and penicillin changes the permeability of the cell membrane and cell wall (Lubitz and Wendisch, 2016; Hashimoto, 2016).

Fermentation is the most economical and eco-friendly means of producing glutamic acid, with low-temperature requirements and the possibility of using cheaper carbon sources such as agricultural residues (Talluri *et al*., 2019), for nearly five to six decades, amino acids are produced through fermentation route (Betemariam and Dawit, 2016; Vijayalakshmi *et al*., 2019).
Hence, the present study is targeted to the determined proximate composition of the acid-treated wheat bran used for glutamic acid production by *Enterobacter* sp. strain BUK_BTE17 MW597320.

MATERIALS AND METHOD

Substrate Collection

About 20 kg of fresh wheat bran (substrate for bacterial growth) was collected from the wheat milling station at Makarfi local government Area of Kaduna state. The wheat bran sample was packaged into a polythene bag, labeled appropriately, and transported to the Department of Microbiology, B.U.K, for analysis.

Apparatus

Apparatus needed are; Analytical balance, Desiccator, Crucible with lid, Thermosetting Oven, and Tongs, metal dishes, Muffled furnace, Thimble, Reflux Condenser, Round bottomed flask, heating mantle, appropriate solvent.

Bacterial strain

The bacterial strain used in this study was ***Enterobacter*** **sp. strain BUK_BTE17 MW597320** isolated from locally produced cheese and its ability to produce glutamic acid was previously studied.

Acid treatment of Wheat Bran

A weighed amount of fresh wheat bran of (50 g) was placed in a 1000 mL Erlenmeyer flask and 250 mL of 1.0 M sulphuric (H_2SO_4) acid was added to the sample. The mixture was autoclaved at 121°C for 30 min and the material obtained after treatment was then filtered through muslin cloth and washed several times under running distilled water until no color is visible in the wash water, a little portion of the treated biomass was dried in an oven at 70ºC for 24 h and was ground to fine particle size in a laboratory mill for the proximate analysis (Bishir *et al*., 2016).

Proximate composition Analysis of Acid-treated wheat bran

The proximate analysis was conducted according to the Association of Analytical Chemists (AOAC, 2005).

Determination of Moisture Content

Moisture content determination is a crucial factor that is critical in food quality, preservation, and resistance to deterioration. The moisture content of food is often determined by a variety of methods.

Procedure

Washed crucibles were oven-dried at 105^0C for an hour to make sure total dryness. They were then transferred into the desiccator to cool for about 30 min. The crucibles were weighed, and the weight was recorded as (W1). A sample (5 g) of wheat bran was weighed into the dried pre-weighed crucible (W2). The crucibles and the content were oven-dried at 105^0C for 4 h. The sample was removed from the oven and dried until a constant weight is obtained. After drying, the crucible was transferred into the desiccator to cool for about 45 min and weighed (W3). This analysis was carried out in triplicate and the average value was recorded as moisture content.

Calculations

(%) Moisture content = Loss in weight due to drying

Weight of sample before drying

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

W□ = Weight of empty Crucible, W□ = Weight of empty crucible + sample before drying

W□ = Weight of crucible+ sample after drying (constant weight).

Determination of Ash Content

Ash is the inorganic residue after the water and organic matter have been removed from a substance by heating in the presence of certain oxidizing agents.

Procedure

A clean crucible was pre-dried in an oven for 30 min at 100^0C to assure total dryness of the crucible, and then transferred into the desiccator to cool for 30 min and weighed in an electronic weighing machine as W1. A (5 g) of the sample was weighed into it and weighed as W2. It had been placed in a muffle furnace for 4 h and the temperature was slowly increased to 450˚C to avoid incomplete ashing. The sample was ash until it becomes whitish. It was removed into the desiccator with a tong and cooled to temperature for an hour. The sample was reweighed as W3. The percentage ash was calculated as followed and the average taken:

Calculation: =

% Ash content = $weig□tofas□$ - $weig□tofsample\ (afterdrying)$∗100

$$\% \text{ Ash} = \frac{W3 - W1}{W2 - W1} \times 100$$

Determination of Crude Fat

Fats are defined as mixtures of varied glycerides of fatty acids, which are

sparingly soluble in water and a few certain organic solvents.

Principle
Principle, heating with concentrated HCl dissolves the fat and other materials; the fat is then extracted with ether. This method is named the Werner Schmid method.

Procedure
Previously dried, the fat-free thimble was weighed as W1. A (3 g) of the sample was weighed into the thimble and weighed as W2. The thimble and the sample were carefully wrapped and tied. Washed and dried 500 mL round bottom flask was weighed as W3. The flask was half crammed with 40/60 petroleum ether and the sample was dropped into the sample holder of the soxhlet extraction apparatus. The Soxhlet extractor was fitted with a condenser, the flask was then placed on a heating mantle and the heat source was adjusted to permit it to boil gently at 34^0C. It was allowed to siphon for over 3hrs. The condenser was detached, and the thimble was removed. Petroleum ether was distilled from the flask. The distilling flask containing the oil was air-dried at 100^0C for 5 min to get rid of the solvent residues in the oil. This was put inside a desiccator to cool and the weight was taken as W4. The proportion of fat contained was determined thus:
% Crude fat = $Weig\square t$ of Flask + oil – Weight of empty flask

$InitialWeig\square tofSample$ * 100 (%)
Crude Fat = $W4-W3$ $W2-W1$*100

Determination of Crude Fibre Content
Crude fibre is the portion of the plant material that is not ash. The fibre was originally thought to be bad and the indigestible portion of foodstuffs. However, the advantages of a diet high in fibre are discovered. Foods with high fibre content can absorb cholesterol and toxic agents in food.

Principle
After boiling the samples with sulfuric acid and sodium hydroxide, the un-dissolved residue is separated by filtration. Weighed and ashed the loss of weight results from ashing similar to the crude fibre present in the samples.

Procedure
The starch and also the protein part of the acid-treated wheat bran were dissolved by boiling with acid then with a very strong base (NaOH). The residue, which consists of cellulose and lignin was washed, dried, and weighed. The residue is ashed and the weight is subtracted from the load of the residue. The defatted sample (2 g) was weighed (W1) into a 250 mL beaker containing 200 mL of 0.125 M tetraoxosulphate (iv) acid (Sulphuric acid). The mixture was heated in a steam bath at 70 – 90^0C for 2 h, it had been allowed to cool down. The cooled mixture was filtered using a muslin cloth over a Buckner funnel. The residue was washed three times with hot water to get rid of the acid then put in a beaker containing 200 mL of potassium hydroxide. The mixture was heated as before over a steam bath for 2 h. and was filtered, and the residue was washed 3 times with hot water, then with petroleum ether and water. The ultimate residue obtained was put in a clean pre-weighed (W2) crucible and dried at 120^0C to a constant weight. The crucible with the oven-dried sample was put in a muffle furnace and ashed at 550^0C for 30 min, the sample became ash white. The crucible and its contents were removed from the furnace, cooled in a desiccator, and reweighed (W3).

Percentage fibre was calculated as followed:
% Crude fibre = weight of oven-dried sample – weight of ash X 100

Initial weight of sample, % Crude fiber = $W2-W3/W1*100$

Determination of Crude Protein

Proteins are necessary nutrients for the human body. They are one of the building blocks of body tissue, and also function as a fuel source. It involved three stages.

Digestion/hydrolysis

Hydrolysis is the decomposition of organic nitrogen into ammonium sulphate.

A (0.15 g) of the Acid Treated Wheat bran was placed into a Kjeldahl flask and a catalyst was added followed by 15 mL of concentrated sulphuric acid. The tubes were assembled in a digestion box and the fume cupboard closed. The content of every tube was heated until the solution assumes a green color then cooled after which each digest was transferred with several pieces of washing into 100 mL with water.

Distillation/Neutralization

Neutralization is the conversion of $(NH_4)_2SO_4$ into ammonium gas.

The digest was first steamed through the Markham distillation apparatus for about 15min. Under the condenser, a 100 mL Erlenmeyer flask containing 10 mL of 4% boric acid was placed and two drops of the indicator were added. Ten millilitres of 40% NaOH was added to 10 mL of the digest after which the NaOH-sample mixture was distilled, and the distillate was collected in the boric acid-indicator mixture until it turned green. This was then titrated with N/100 (0.01N) acid.

Titration

Titration is the quantity of ammonia that is been absorbed in the receiving solution during distillation. The solution in the receiving flask was titrated with 0.01 N hydrochloric acid, The Nitrogen content and hence the protein content of the sample was calculated as follows: The percentage (%) protein content was then calculated as; Final reading – Initial reading X standard number of nitrogen (1.4) divided by initial weight X standard protein factor (6.25)

Protein (%) = (Vs – Vb)/ W x 6.25 x 1.4

Where: Vs = titre reading of the sample, V2 = titre reading of the blank, W = Initial weight of the wheat bran**.**

Determination of Carbohydrate

By difference, in this method, carbohydrate content was obtained by calculations having estimated all other fractions by proximate analyses i.e. CHO = 100- (% moisture + %Ash + %fiber +%fat+ %protein).

Screening Medium for Glutamic Acid Production

The compositions of the screening medium used for glutamic acid production per 1000 mL of distilled H_2O is as follows; glucose, 5 g; calcium carbonate, 1 g; ammonium sulphate, 1 g; potassium di-hydrogen phosphate, 0.3 g; di-potassium hydrogen phosphate, 0.7 g; magnesium sulphatehepta-hydrate, 0.01gm; ferrous sulphatehepta-hydrate, 0.2 mg; magnesium chloride tetrahydrate, 0.2 mg; and d-biotin, 100 µg (Hadia *et al.*, 2012).

Culture procedure

Screening medium (100 mL) was taken in a 250 mL sterilized flask and inoculated with 24hrs old bacterial broth culture. After inoculation, the flask was incubated on a shaker incubator at 30ºC for 72 h at 150 rpm. The sample was taken after 72hrs and centrifuged at 10,000rpm for 10 min. Supernatants were examined for glutamic acid (Betemariam and Dawit, 2016).

Qualitative Determination of Glutamic Acid

Qualitative analysis for glutamic acid was done by thin-layer chromatographic technique TLC, (Nasab*et al.*, 2007). For TLC, Silica gel G was used having dimensions 5x4 cm was used, spotted with

standard glutamic acid solution and solution from the supernatant of the samples, 0.5 cm above from the bottom by means of a micro-capillary tube. At room temperature, spots were air-dried. The TLC plate was then dipped up to 0.5 cm in a solvent system having 3 nButanol, 1.5 mL acetic acid and 1 mL distilled H_2O (3:1.5:1). By using an ascending method, spots were developed by allowing the solvent to run 30 min, up to 4.5 cm from the bottom. After air-drying, the TLC plate was sprayed uniformly with 0.2% ninhydrin in (95%) acetone. The TLC plate was then placed in an oven set at 65ºC for Ten min after air-drying. Colored spots of amino acids were produced which indicated the positions of amino acids present in the sample(s). Through these well-defined colored spots, the retention factor (RF) was also calculated and the glutamic acid present in the unknown samples was identified by comparing with the standard glutamic acid colored spot based on their RF. The retention factor was calculated using the following formula; RF = Distance moved by the spot /Distance moved by the solvent (No unit).

Quantitative Determination of Glutamic Acid

For quantitative estimation of the amino acid produced, 2 mL of the supernatant from each of the fermented screening mediums was taken separately in test tubes and 100 uL of 0.5% ninhydrin in acetone was added and heated for 15 min in a boiling water bath. The tubes were then cooled to room temperature and glutamic acid was quantitatively estimated by taking readings at 570 nm using a spectrophotometer with reference to the standard curve (Nasab *et al*., 2007; Betemariam and Dawit, 2016).

Standard Curve of Glutamic Acid

To estimate glutamic acid quantitatively by spectrophotometry method as described by Shagufta (2014), the standard curve was firstly plotted between various diluted concentrations (0.1 g/L -0.5 g/L) of standard glutamic acid and their respective optical densities (OD) at 570 nm.

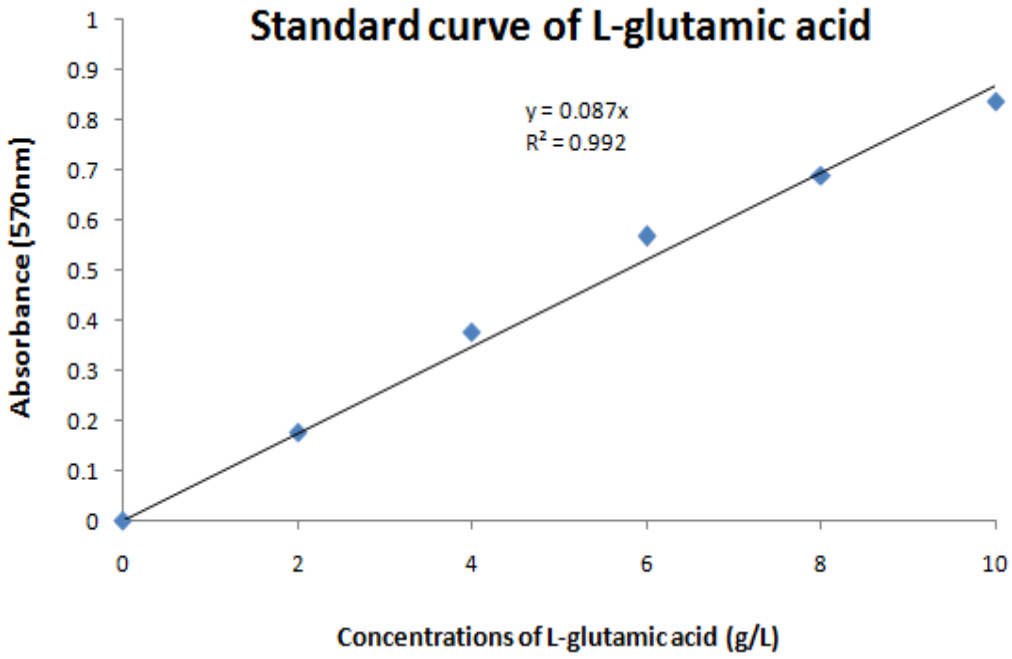

Figure 1: Standard Curve of Glutamic Acid

Glutamic Acid Production from Wheat Bran by Submerged Fermentation

Basal Medium

For glutamic acid production by *Enterobacter* sp strain, BUK_BTE17 MW597320through submerged fermentation. The basal salt medium consists of the following per litre: 5 g, Yeast extract, 5 g $(NH_4)_2SO_4$; 5 g, urea; 2 g, KH_2PO_4; 2 g, K_2HPO_4; 0.25 g, $MgSO_4 \cdot 7H_2O$; 0.01 g, $FeSO_4 \cdot 7H_2O$; 0.01 g, $MnSO_4 \cdot 5H_2O$; 0.03 mg, $ZnSO_4 \cdot 7H_2O$; 100 µg of biotin. The initial pH was adjusted to 8.0 with 1 N NaOH (Hadia *et al*., 2012).

Shake Flask Fermentation

Fifty millilitres of the basal medium was mixed separately with 10 g powdered acid-treated wheat bran in 250 mL Erlenmeyer flasks and labelled appropriately. 24 hr-old culture of isolates was added and incubated on a rotary flask shaker (150 rpm) at 30°C for 72 h (Nasab *et al*., 2007). The qualitative and quantitative analyses of the glutamic acid produced were carried out and results were recorded accordingly.

RESULTS

Proximate Compositions of the Acid Pre-treated Wheat bran

As shown in Table 1 below, the proximate composition analysis of the powdered acid treated-wheat bran sample revealed the Moisture content of 4.27±0.090%, Ash content of 16.43±0.087%, Crude fiber 14.57±0.095%, Crude fat 4.02±0.029%, Crude protein 2.69±0.093%, and Carbohydrate content of 58.03±0.051%, respectively.

Table 1: Proximate Compositions of Acid-treated Wheat bran

Component	Proximate Compositions (%)
Moisture	4.27±0.090
Ash	16.43±0.087
Crude Fiber	14.57±0.095
Crude Fat	4.02±0.029
Crude Protein	2.69±0.093
Carbohydrate	58.03±0.051

Screening for Glutamic Acid Production

The isolate *Enterobacter* sp. strain BUK_BTE17 MW597320 screened was found to be capable of glutamic acid production. The glutamic acid was identified qualitatively by thin-layer chromatography, with a retention factor (RF) of 0.5 which is the same for the standard glutamic acid indicating good precision of the method used. The isolate gave the maximum (0.98 g/L) glutamic acid yield during screening before the final productions by submerged fermentation of acid-treated wheat bran, the amount of glutamic acid was determined spectrophotometrically at 570nm and quantified using the standard curve.

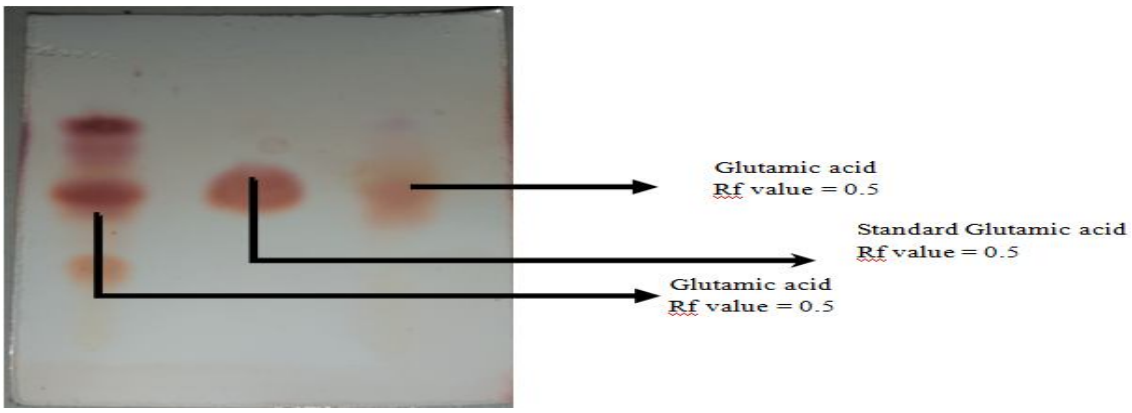

Figure 2: Spots of Glutamic Acids on TLC plate

DISCUSSION

Industrial products have been produced as metabolites by numerous microorganisms. Glutamic acid production by employing living cells is more appreciable for industrial purposes (Fahimitabar *et al*., 2021). However, the consistency may be as a result of both studies utilizing 1.0 M $H_2SO_4$for substrate treatment. Treatment of the wheat bran enhanced glutamic acid production by virtue of its high carbohydrates, high ash (mineral salts), and an appreciable amount of protein (nitrogen source) contents that are required by wild bacteria for growth and glutamic acid production. This might be accountable based on the fact that acid pretreatment of substrate usually does not remove all the lignin from the substrate, but sometimes only modifies the lignin-carbohydrates linkage as reported by Damisa *et al*. (2008). Similarly, the work of Bishir *et al*. (2016) stated that acid treatment of substrate may even permit cellulose to re-anneal, resulting in the horrification of cellulose into microfibrils instead of increasing the surface area of the wheat bran. In a related finding the lower carbohydrates contents of acid-treated (HCl) hydrolysate were reported by Nasab *et al*. (2010) where a lower content of carbohydrates was obtained from HCl-treated hydrolysate than that from H_2S0_4-treated wheat bran (20.56% and 58.03%) removal of lignin. Overall, the recalcitrant nature of the lignocellulose substrates used for microbial utilization as an energy source becomes a hindrance to its industrial utilization, thus, pretreatment is needed to extend the surface area of cellulose by removing the lignin seal, solubilizing the hemicelluloses, disrupting crystallinity, and increasing the pore volume then making the wheat bran substrate amenable for microbial utilization or enzymatic hydrolysis for improved glutamic acid production by wild bacteria isolate. The wheat bran was high in carbohydrate content hence is often a good source of energy. Carbohydrates are good sources of energy. However, the energy is stored as glycogen, which is the reservoir for glucose and can be used in the industrial production of glutamic acid (Bishir *et al*., 2016).

Glutamic acid concentration of (0.98 g/L) agrees with the work of Hadia *et al*. (2012) who reported a glutamic acid concentration of 1.5g/L after screening. This agreement could also be as a result of both pieces of research, utilizing biotin at a concentration range of (50 µg to 100 µg). In a related study, a lower concentration (0.25 g/L) of glutamic acid was reported by Bishir *et al*. (2016) differences could also be as a result of much higher concentrations of biotin used (200 µg) in the study. Microorganisms require biotin for cell growth.

The study by Betemariam and Dawit, (2016) showed that maximum glutamic (92.53±.23 g/L) was produced in a medium with glucose as a carbon source. In the current study, a maximum glutamic acid yield of (11.30 g/L) was produced by isolate. The presence of glucose in the fermentation medium is not enough for the excellent production of glutamic acid, but the concentration of glucose should be taken into consideration because the growth of microorganisms is often hindered by glucose concentration in the medium (Betemariam and Dawit, 2016). Khan *et al*. (2013) reported that the growth of*C. Glutamicum* was limited by the substrate (glucose) up to the initial glucose concentration of (50 kgm^{-3}). Hassen *et al*. (2008) reported that 10% glucose is suitable for glutamic acid production which is consistent with this study. For any fermentation process choice of substrate is the most important and basic requirement for microbial biosynthesis, an inexpensive substrate with multiple nutrients is ideal for industrial biotechnological processes (Betemariam and Dawit, 2016).

CONCLUSION

The use of locally available and nutrient-rich raw material (wheat bran) as a substrate for glutamic acid production is possible and makes the process economical and in turn reduces the environmental pollution by biotransformation to commercially useful bioproducts. Treatment of the wheat bran enhanced glutamic acid production by virtue of its high ash (mineral salts) and an appreciable amount of protein (nitrogen source) contents that are required by wild bacteria for growth and glutamic acid production. Different agricultural residues such as potato peels, sugar cane bagase, and cereal husk should also be tried for their potential to serve as substrates for glutamic acid production.

Acknowledgements: This research was not supported by any grant but was self-funded research.

Author Contributions: Aliyu Shuaibu supervised its conduct. Hamza Magaji carried out the experiments wrote and compiled the manuscript, while Eberemu, Nkiru Charity, and Umar Ismail participated in the supervision process. All authors checked and supported the final manuscript.

Conflicts of Interest: The authors declare no conflicting interest.

REFERENCES

AOAC. (2005). *Official Method of Analysis.* Gaithersburg, USA: Association of Official Analytical Chemists.

Betemariam, K., & Dawit, A. (2016). Production of L- Glutamic Acid From Wild Bacterial Isolates From Soil Using Batch Fermentation. *International Journal of Innovative Pharmaceutical Sciences and Research*, 1103-1114.

Bishir, M., Alhaji, S. A., & Obansa, I. A. (2016). Glutamic Acid Production from Rice Husk Using Corynebacterium glutamicum Isolated from Soil. *American Journal of Bioscience and Bioengineering, 4* (6), 70-76.

Biswas, P., Bharti, A. K., Kadam, A., &Dutt, D. (2019). Wheat bran as a substrate for enzyme production and its application in the bio-deinking of mixed office waste (MOW) paper. *BioResources*, *14*(3),5788-5806.

Damisa, D., Ameh, J.B. and Umoh, V.J. (2008). Effect of Chemical Pretreatment of Some Lignocellulosic Wastes on the Recovery of Cellulase from *Aspergillusniger*AH3 Mutant. *African Journal of Biotechnology*, **7**(14):2444-2450.

El-Shishtawy, R. M., Mohamed, S. A., Asiri, A. M., Abu-bakr, M. G., Ibrahim, I. H., & Al-Talhi, H. A. (2014). Solid fermentation of wheat bran for hydrolytic enzymes production and saccharification content by a local isolate Bacillus megatherium. *BMC Biotechnology*, *14*(1), 1-8.

Fahimitabar, A., Razavian, S. M. H., &Rezaei, S. A. (2021). Application of RSM for Optimization of Glutamic acid Production by Corynebacteriumglutamicum in bath culture. *Heliyon*, e 07359.

Hadia, G., Abdul Haleem, S., & Nabila, Y. (2012). Fermentative Production of Glutamate by Newly Isolated Soil Bacteria. *International Journal of Pharmaceutical & Biological Archives, 3* (6), 1368-1376.

Hashimoto, S. I. (2016). Discovery and history of amino acid fermentation. *Amino Acid Fermentation*, 15-34.

Hassan, E. G., Alkareem, M. A., & Mustafa, M. I. (2008). Effect of Fermentation and Particle Size of Wheat Husk on the Antinutritional Factors and Bread Quality. *Pakistan Journal of Nutrition*, 521-526.

Khan, S. H., Rasool, G., & Nadeem, S. (2006). Bioconversion of Cane Molasses into Amino acids. *Pakistan Journal of Agricultural Sciences, 43* (3-4), 156-161.

Khan, S. N., Singh, R. P., & Prasadc, B. (2013). Studies on substrate inhibition in the microbial production of L-glutamic acid. *International Journal of Engineering Research & Technology, 2*, 1-7.

Lubitz, D., &Wendisch, V. F. (2016). Ciprofloxacin triggered glutamate production by Corynebacteriumglutamicum. *BMC microbiology*, *16*(1), 1-12.

Nakayama, Y. (2021). Corynebacteriumglutamicummechanosensing: From osmoregulation to L-glutamate secretion for the avian microbiota-gut-brain axis.*Microorganisms*, *9*(1), 201.

Nasab, M. M., Masoumeh, I., & Sara, H. (2010). Glutamic Acid Production from Potato by Brevibacterium linens. *International Journal of Nutrition and Food Engineering*, 610-612.

Nasab, M. N., Ansaris, S., & Montazer, Z. (2007). Fermentative Production of Glutamate by Corynebacterium glutamicum from Different Carbon Sources. *Journal Iranian Agricultural Resources*, 99-105.

Shafaghat, H., Najafpour, G. D., Rezaei, S. P., & Sharifzadeh, M. (2010). Optimal Growth of Saccharomyces cerevisiae (PTCC 24860) on Pretreated Molasses for the Ethanol Production: the Application of the Response Surface Methodology. *Journal of Chemical Industry and Chemical Engineering, 16* (2), 199-206

Shagufta, I. (2014). *Bioconversion of Agricultural Wastes to Glutamate and its Biological Evaluation in Broiler Chicks.* Lahore, Pakistan: PhD. Thesis Submitted to the Department of Biochemistry, Faculty of Biosciences, University of Veterinary and Animal Sciences.

Shyamkumar, R., Moorthy, I. M., Ponmurugan, K., & Baskar, R. (2014). Production of L-glutamic Acid with Corynebacterium glutamicum (NCIM2168) and Pseudomonas reptilivora (NCIM 2598): A Study on Immobilization and Reusability. *Avicenna Journal of Medical Biotechnology*, 163-168.

Talluri, P. V., Lanka, S. S., & Saladi, R. V. (2019). Statistical Optimization of Process Parameters by Central Composite Design (CCD) for an Enhanced Production of L-asparaginase by Myroides

gitamensis BSH-3, a Novel Species. *Avicenna Journal of Medical Biotechnology, 11* (1), 59-65.

Vijayalakshmi, P., Vaddadi, U., Reddy, K., & Manasa, R. V. (2019). Fermentative Scale-up Production of L-Glutamic Acid Using Response Surface Methodology. *International Journal of Pharmaceutical Science Revised Resource, 58* (1), 27-33