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Optimization of Glucose-Isomerase (GI) Enzyme Activity on Ripe Jackfruit Pulp (*Artocarpus heterophyllus*) to Produce High Fructose Sugar

Noopur Gautam[1], Manoj Kumar[2], Shiv Kumar[3], Uzma Siddiqui[4]

[1]Institute of Food Technology, Bundelkhand University, Jhansi, Uttar Pradesh, India
[2,3] Institute of Food Technology, Bundelkhand University, Jhansi, Uttar Pradesh, India
[4]Institute of Food Technology, Bundelkhand University, Jhansi, Uttar Pradesh, India

Corresponding Author: Noopur Gautam

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ABSTRACT

Glucose-isomerase activity was optimized on ripened jackfruit pulp (*Artocarpus heterophyllus*). The GI action on 20% pulp extract of ripe jackfruit was investigated after it was analyzed for fructose content, reducing sugar, non-reducing sugar and total sugar which were found 7.53 g/100g, 11.56 g/100g, 5.10 g/100g and 16.93g/100g respectively. The GI activity was found bi-directional at pH 7.0, temperature 70˚C, time 120 min on 5 ml substrate with 100 mg of GI concentration. The negative value (-0.08 µmoles/min) of GI activity on 20% pulp extract indicated the reverse reaction and was found dependent on the concentration of glucose and fructose present in the jackfruit pulp which further confirmed by enriching the pulp with 10% glucose and 10% fructose. Due to the reverse direction of GI activity on jackfruit pulp, the fructose was separated from the pulp before the application of GI, leaving glucose for isomerization to get high fructose sugar.

Keywords: Jackfruit; Glucose-isomerase; Glucose; Fructose; High fructose sugar.

Highlights

1. Optimization of GI enzyme on ripe jackfruit pulp was done to produce High Fructose Sugar.
2. GI activity was found dependent on concentration of glucose to fructose ratio in jackfruit pulp.
3. The GI activity was observed in reverse direction (negative) on jackfruit pulp.
4. Removal of fructose sugar was required initially for GI activity to produce High Fructose Sugar.

Practical Application

This study will help to identify sugar rich jackfruit varieties and optimize the use of GI enzyme for extraction of high fructose sugar which can be commercially exploited in food and pharmaceutical industries for developing products with better quality and stability. Production and extraction of High Fructose Sugar from ripen jackfruit is likely to valorize this under-utilized fruit and encourage cultivation of jackfruit in larger, also ensuring better profits to growers, processors and consumers.

INTRODUCTION

Jackfruit is one of the most wonderful and largest edible fruits that has opened the door to get exploited for mankind. It is delicious tropical composite fruit with succulent and firmly textured bulbs (Saxena et al., 2009) belonging to family *Moraceae*. Jackfruit is a climacteric fruit which is harvested after maturity and ripening is followed thereafter

due to increased production of ethylene (Singh et. al., 2000). Being highly cross pollinated and mostly seed propagated, it exists in innumerable types or forms, with different fruit characteristics. Fruits differ among themselves in the color, sweetness, acidity, flavor and taste of flakes(Jagadeesh et al., 2007). The fruit deteriorates rapidly upon ripening like other climacteric fruits(Saxena et al., 2009)because in climacteric fruits, carbohydrates are accumulated during maturation in the form of starch and as the fruit ripens starch is broken down into sugars (Dhillon et.al., 2002). Jackfruit is consumed both as a vegetable in the unripe stage and also as a fruit when ripe but its strong disagreeable odor and aroma resembling that of decaying onions keeps people away from accepting as a regular fruit like pineapple and banana. Owing to excellent processing qualities, various value-added products are made from jackfruit like chips, pickles, brined jack, RTC, candy, nectar, jam, rind jelly, fruit leather, squash and RTS (Srivastava et al., 2017) and osmotic dehydrated jackfruit preserve(Rahman et al., 2012). According to agricultural experts, market glut and waste accumulation is the major setback in jackfruit processing hence there is a need to focus on other lines simultaneously like extraction of various components from jackfruit and its waste such as β-carotene, dietary fiber, pectin, lectins, sugars and jacalin etc. Since ripe jackfruit contains a very high number of simple sugars, it can be a good source of invert sugar and high fructose sugar to be used in confectionary, bakery and pharmaceutical industries. According to (Chaira et al., 2010) high fructose sugar has more desirable functional properties such as increased osmotic dehydration, high solubility and preventing crystallization in food products.

Hence, keeping in view, the study was planned as an attempt to convert natural sugar present in the ripen jackfruit bulbs into high fructose sugar through Glucose-isomerase enzyme. This present work included optimization of commercial Glucose-isomerase (GI) on artificial substrates (similar to sugars present in ripen jackfruit) and jackfruit pulp, converting it into fructose rich sugar.

MATERIAL AND METHODS

Materials:

The raw material required to carry out investigation was ripened jackfruits. The jackfruits were procured from Kerala, Bihar and Uttar Pradesh. The cultivated as well as local varieties were collected. From Kerala, two local varieties namely *Koozha-chakka* and *Varika* were taken, from Siwan (Bihar) a local variety collected was denoted as BS1(Bihar Siwan1). From U.P. Faizabad two cultivated varieties from NDUA&T (Narendra Dev University of Agriculture and Technology) was collected which were named NJ11 and NJ14. All five varieties were selected for the screening of sugars in the fruit. The jackfruits were harvested at the maturity and were kept in dark, dry and closed room for seven days (Shamsuddin et al., 2009). After seventh day i.e., on day 8^{th}, the fruits were cut open and the pulp was taken for analysis and experiments.

Other materials used:

Glucose-isomerase- The commercial name to this enzyme is Sweetzyme® T special. The declared activity of this enzyme was Glucose-isomerase. It was procured from Novozymes South Asia, Bangalore. The Sweetzyme® T special was immobilized granulate having chocolate brown color with slight fermentative odor.

Resins- For chromatographic separation of sugars, styrene-DVB gel matrix-based resin known as Dowex™ Monosphere ™ 99 Ca/320 was used. This resin was procured from The Dow Chemical Company and supplied by Supelco of Germany.

Instruments used:

Spectrophotometer- GENESYS 10 UV spectrophotometer

High Speed Centrifuge- CT14RD cooling centrifuge

Water Bath- Water Bath with temperature range 30˚C- 190˚C.

Hand Refractometer.

Method:

Sugar estimation in Ripen Jackfruit of selected varieties-The parameters studied for sugar estimation were: TSS, Total sugar, Reducing sugar and Non-reducing sugar. The variety with highest sugar content was selected for further investigation.

Determination of Sugars- The test method adopted for TSS was Rangannna (1979) and the values were expressed in percentage, Total sugar by Anthrone method and Reducing sugar was estimated by DNS method in percentage (%) (Swahney and Singh, 2008). Non-reducing sugar was calculated by the formula (as given below) mentioned by Mishra, 2004.

% Non-reducing sugar = 0.95(% Total sugar - % Reducing sugar)

Fructose sugar was estimated at 450nm wavelength using Seliwanoff's test (Test for ketoses) mentioned by Swahney and Singh, 2008.

Sample Preparation:

Extraction of Pulp- The technique of extracting pulp was described by Mishra (2004). The technique was used with slight modification. The ripe fruit was cut open into two halves. The core was removed and the carpels (bulbs) from the fibrous mesocarp were taken out. The seed with seed coat was removed and the carpel was cut into pieces. The flesh (carpel pieces) was mixed thoroughly with water at the ratio of 4:1(flesh to water). The mixture was blended (pulped) into blender and then stored in jar under -20°C temperature.

Pulp Solution- To carry out studies, 100g pulp was diluted with 500ml distilled water to make Pulp Extract of 20%. It was centrifuged for 10minutes at 10,000 rpm at 20°C to remove all insoluble cell debris. The aliquot taken was denoted as Pulp Extract (PE) and was used for all further experiments.

Deproteination of Pulp Extract- The pulp extract was deproteinated to get rid of all proteins present in the pulp of jackfruit which might interfere in the enzymatic conversion of sugars present in the fruit. Around 50 ml of pulp extract was taken and centrifuged in High-Speed centrifuge for 45 min at 10,000 rpm at 20°C.The aliquot filtered was used for further experiment.

Estimation of Sugars in Pulp Extract- Amount of Fructose, reducing sugar, non-reducing sugar and Total sugar was estimated through the above-described methods (2.2.2).

Optimization of Enzymatic activity:

Sweetzyme® T Special (Glucose-isomerase)

The enzyme activity of glucose-isomerase was studied on different formulations of substrate concentration, time and enzyme concentration (Bahramian et al., 2011). Ghosh et al., 2017 carried out optimization of commercial enzymes on the basis of physiochemical, nutritional property and sensory quality for extraction of Jamun Juice.

Optimization of Different Substrate Concentration- The optimum substrate concentrations of various formulations of different monosaccharide were tested. This was based on the composition of these sugars found in the ripened jackfruit (Samaddar, 1990).

1. The first combination was – 10g of Glucose, 2g of Fructose, 2g of Galactose, 2g of Sucrose and 1g of Arabinose. All the sugars were dissolved in 100ml of distilled water. This combination was denoted by AS1.
2. The second combination was – 10g of Glucose, 2g of Galactose, 2g of Sucrose and 1gof Arabinose. This combination was denoted by AS2.
3. The third combination was –10g of Glucose, 2g of Galactose and 1g of Arabinose. This mixture was denoted by AS3.
4. The fourth combination was – 10 g of Fructose, 2 g of Galactose and 1 g of Arabinose. This was denoted as AS4.
5. The fifth combination was – 5 g of Glucose, 5 g of Fructose, 2 g of Galactose and 1g of Arabinose. This combination was denoted by AS5.
6. The sixth combination was – 2g of Glucose, 8gof Fructose and 2 g of

Galactose. The combination was denoted by AS6.

7. The seventh combination was – 8g of Glucose, 2 g of Fructose and 2 g of Galactose. The combination was denoted by AS7.

A) Experimental design for GI activity on Artificial Substrate

Artificial substrates	Phosphate buffer (ml)	Distilled water (ml)	GI enzyme (mg)	Amount of Artificial substrate (ml)	Total volume (ml)
AS 1 + E	10	5	100	5	20
AS 1 - E	10	5	-	5	20
AS 2 + E	10	5	100	5	20
AS 2 - E	10	5	-	5	20
AS 3 + E	10	5	100	5	20
AS3- E	10	5	-	5	20
AS 4 + E	10	5	100	5	20
AS 4 - E	10	5	-	5	20
AS 5 + E	10	5	100	5	20
AS 5 - E	10	5	-	5	20
AS 6 + E	10	5	100	5	20
AS 6 - E	10	5	-	5	20
AS 7 + E	10	5	100	5	20
AS 7 – E	10	5	-	5	20

The activity of enzyme on different combination of sugar was measured by the amount of fructose produced (mg). This amount of fructose was estimated by Seliwanoff's method (Swahney and Singh, 2008). The pH was set at 7.0 and temperature was maintained 70°C (Deshpande & Rao, 2006) and (Parisa et al., 2008).

Optimization of Reaction Time for Enzyme (Glucose-isomerase)- Optimum time required for the reaction to complete was studied on substrate AS3. The reaction time was measured by the maximum amount of fructose produced. The fructose content in turn was measured by Seliwinoff's method. The time observed was every 30 minutes for 2 hrs., i.e., 30min, 60min, 90min and 120min. The pH was set at 7.0 and temperature was maintained 70°C.

Optimization of Enzyme (Sweetzyme® T Special) Concentration- To optimize enzyme use, the different concentration of enzyme was taken i.e., 5mg, 25mg, 100mg and 400mg. The reaction was carried out for 2 hrs. and evaluated by the amount of fructose produced. The pH was set at 7.0 and temperature was maintained 70°C.

Optimization of Substrate concentration- The optimum amount of substrate required for the conversion reaction to happen was studied by using various quantities of AS3 substrate i.e., 10ml, 5 ml, 2 ml and 1 ml of mentioned substrate solution for 2 hrs. with enzyme concentration 100mg.The pH was set at 7.0 and temperature was maintained 70°C.

Activity of Glucose-isomerase (Sweetzyme® TS) on Pulp Extract- The activity of Glucose isomerase on different concentration of Pulp Extract was studied. The experiment was designed by taking 7 conical flasks of 250 ml considering as 7 sets. In each set, enzyme, phosphate buffer, distilled water and substrate was added as below:

B) Experimental design for GI activity on Pulp Extract.

Phosphate Buffer (ml)	Enzyme in mg (Sweetzyme)	Substrate in ml (Pulp Extract)	Distilled water (ml)	Total Volume (ml)
10 (B)	100	0.0	10	20
10	100	0.2	9.8	20
10	100	0.5	9.5	20
10	100	1.0	9.0	20
10	100	2.0	8.0	20
10	100	5.0	5.0	20
10 (C)	00	5.0	5.0	20

The pH was set at 7.0 and the sets were sealed with aluminium foil. These 7 sets were kept in water bath maintained at temperature 70°C for 2 hrs. After 2 hrs. of reaction time the sets were taken out cooled and then tested for fructose content in (Seliwinoff's method) triplicate to measure the activity of Glucose isomerase (Sweetzyme®).

Activity of Glucose-isomerase on Pulp Extract enriched with reducing sugar- Enrichment of Pulp Extract with 10 % glucose and 10 % fructose was done. In 20 ml of pulp extract (20% of pulp), 2g of dry glucose powder was added which was denoted by ANS 1 and then the activity of glucose-isomerase was measured, while in another set of experiment, 20 ml of pulp extract (20% of pulp), 2g of dry fructose powder was added which was denoted by ANS 2 and then the activity of glucose-isomerase was measured. Four sets were prepared as designed below.

C) Experimental design for GI activity on enriched Pulp Extract with Glucose (ANS 1) and Fructose (ANS 2)

Samples	Phosphate Buffer (ml)	G I Enzyme in (mg)	Substrate in ml (Pulp Extract)	Distilled water (ml)
ANS 1 + E	10	100	5	5
ANS 1 - E	10	-	5	5
ANS 2 + E	10	100	5	5
ANS 2 - E	10	-	5	5

ANS1 + E = Enriched pulp (10 % glucose) with enzyme GI,
ANS1 – E = Enriched pulp (10 % glucose) without enzyme GI.
ANS2 + E = Enriched pulp (10 % fructose) with enzyme GI,
ANS2 – E = Enriched pulp (10 % fructose) without enzyme GI.

The pH was 7.0 and the temperature was at 70°C in water bath. After 2 hrs. of reaction time the sets were taken out cooled and then tested for fructose content (Seliwanoff's method) in triplicate to measure the activity of Enzyme (Sweetzyme®).

Chromatographic separation of Glucose and Fructose from Pulp Extract- For the separation of fructose from the mixture of glucose, fructose and other monosaccharaides, ion exchange resin commercially known as Dowex Monosphere99Ca/320 was used. Ion exchange application was performed through batch technique. In batch operation, the resin was agitated in a vessel with the solution to be treated.

Preparation of High Fructose Sugar- The fractions of the elution after undergoing isomerization were collected in the glass beaker (borosil) of 500 ml and for every 100 ml of fructose rich sugar solution 3 g powdered activated carbon (ion exchange) was added and heated in dry oven at 80°C for 60 minutes. The solution was taken out and filtered using Whatman filter paper no.1 and the filtered solution was vacuum dried at 62°C temperature till the TSS of the solution becomes 60°B.

Statistical Analysis

The experimental design was simple CRD. The analysis of variance (ANOVA) of the data was carried out by the technique of SAS (Statistical Analysis System) 9.2. Significance was tested at level 5%.

RESULT AND DISCUSSION

The present study was carried out in order to optimize the GI activity for producing high fructose sugar from the largest fruit which is still finding its space in food processing industry. The high carbohydrate content makes jackfruit an excellent source for natural sweeteners. Hence, an effort was put to convert the total sugar of jackfruit into high fructose sugar.

In table 1a, the value of TSS of five selected varieties of jackfruit was observed as: 29.80% in *Koozha*, 24.67% in *Varika*, 20.80% in BS1 (Bihar Siwan 1), 10.20% in NJ11 and 17.20% in NJ14. Sadasivam and Neelkanta (1976), Muthulakshmi (2002), Mishra (2004) and Jagdeesh et.al. (2007) also reported TSS to vary from 14.63 to 33% and 19.36% to 36.0% respectively. (Shamsuddin et al., 2009) also mentioned the TSS range of 19.03 to 32.53^{0}B for its J33 cultivar and in contrast, NJ11 was

reported to have lowest (10.20%) TSS. The reducing sugar in five selected varieties were found as 11.98%, 9.12%, 6.81%, 0.95% and 5.20% respectively. In NJ11, the reducing sugar content was found too less (0.95%), while Mishra (2004) reported the 17.0% in the same variety. This can be due to the degree of fruit maturity at the time of harvesting though Muthulakshmi (2002) reported the lowest value of reducing sugar as 1.63%. Non-reducing sugar in selected varieties were found in the range of 1.59% to 1.72% with highest value 1.72% in *Koozha* variety. Earlier, Chan and Hen (1975) reported the non-reducing sugar ranges from 2.28 to 3.06% while Chowdhury et al., 1997 reported 1.49g/100g of sucrose in ripe jackfruit pulp.

Table 1 (a) Sugar content in jackfruits of five selected varieties.

S.NO.	Variety of Jackfruit	TSS (%)	Reducing Sugar (%)	Non-reducing sugar (%)	Total Sugar (%)
1	Koozha	29.80	11.98	1.72	13.80
2	Varika	24.67	9.12	1.67	10.88
3	BS1	20.80	6.81	1.65	8.55
4	NJ 11	10.20	0.95	1.59	2.62
5	NJ14	17.20	5.20	1.64	6.93
(Pr>F: <0.001) LSD at 5%		2.1927	1.1682	0.3239	1.9648

The total sugar in *Koozha*, *Varik*a, BS1, NJ11 and NJ14 varieties were found as 13.80%, 10.88%, 8.55%, 2.62% and 6.93% respectively. Muthulakshmi (2002) and Mishra (2004) found that total sugar varies from 8.16% to 20.77%. NJ11 contained lowest amount 2.62% which was very close to the total sugar present in wild variety of jackfruit as reported by (Sundriyal et al., 2011). The amount of fructose in the jackfruit pulp of *Koozha* variety was found to be 7.53%, reducing sugar as 11.56%, total sugar as 16.93% and non-reducing sugar as 5.10% (Table 1a). Chowdhury et al., 1997 reported the amount of fructose and glucose as 4.53g and 2.06 g per 100g of jackfruit so as (Li et al., 2017) too found the glucose: fructose ratio as 0.9 to 1.2 in their eight varieties from China. Rahman et al., 1995, reported the decrease in the water-insoluble dry matter and increase in water-soluble matter during ripening (660-790 g/kg) which included fructose (73-113 g/kg) and sucrose (95 g/kg). Rahman et al., 1999 also reported the free sugar of jackfruit increases with maturity from 1.5 to 10.5% and the main sugar are glucose, fructose and sucrose (Ong et al., 2006). The difference observed in the values of Table 1a and Table 1b of *Koozha* varieties was considered to be the variations in fruits itself of the same variety. The variations in the sugar content of jackfruits may be due to degree of fruit maturity, cross pollination and plant growth conditions. This wide range of variation existing in nature aids in the selection of superior desirable traits (Jagadeesh et.al., 2010). Moreover, Li et al., 2017, stated that no clear correlation exists between sugar content and types of jackfruits (soft & firm) although the glucose: fructose ratio in fruit depends on ripening stage and were found variety specific.

Table 1 (b) Amount of sugar estimated in jackfruit pulp (per 100g)

Fructose	7.53g
Reducing sugar	11.50g
Non-reducing sugar	5.10g
Total sugar	16.93g

In Table 2, the GI activity was studied on artificial substrate AS1, AS2 and AS3 was observed to be 0.126 µmoles/min in AS1, 3.76 µmoles/min in AS2 and 8.5 µmoles/min in AS3 respectively. The GI activity on AS4 and AS5 was found negative (Table 2). The value observed was -0.36 µmol/min and -0.10 µmol/min which indicated the reversible characteristic of GI i.e., conversion of glucose into fructose and vice-versa. Thus, GI enzyme was found working in both the directions (bi-directional) depending upon the concentration of glucose to fructose ratio. This was supported by (Kovalenko et al., 2008) which reported the reaction of

glucose isomerization, was temperature-independent; the G/F ratio equalled 1.1: 1.0. Parisa et al., 2008, too reported the reversible property of GI enzyme. The inter-conversion of D-glucose and D-fructose by GI enzyme was also observed by Kasumi et al., 1981.The Table 2, also indicated the GI activity in forward direction (+ve) when the amount of glucose was taken more (8g) in the substrate (AS7) while the activity of GI was found in reverse direction (-ve) when the amount of fructose was taken more (8g) in substrate AS6. Hence, the bidirectional property was established. The reaction time of GI was studied for 120 min (Table 3) and the maximum activity of GI was reported during 30 min to 60 min of incubation time (120 min) as shown in Table 3 while Mishra & Debnath, 2002,reported incubation time of 60 min for GI activity at pH 6.5-7.5 and temperature 60°C. Parisa et al., 2008, also reported the reaction time of 60 min at pH 6.0-8.5 for GI activity at temperature range of 30-90°C. Kovalenko et al., 2008 in addition reported the maximum glucose-isomerase activity 2µmol/min/g with increased temperature of 60-80°C. Since the objective was to isomerizes glucose into fructose, GI characterization was studied on AS3. Table 3 indicated that with increase in enzyme concentration, the GI activity increases to 3-folds (3 times). 100 mg of GI was selected on the basis of optimum size and limiting amount of GI enzyme available. Mishra & Debnath, 2002 used 40 mg of GI for 0.2 ml of 1M D-glucose. Table 3 showed the GI activity is directly proportional to the amount of substrate AS3.

Table 2. GI activity on artificial substrates at pH 7.0 and temp. 70°C.

S. No.	Artificial substrates	Phosphate buffer (ml)	Distilled water (ml)	GI enzyme (mg)	Amount of Artificial substrate (ml)	Amount of Fructose produced in 120 min due to GI activity at 70°C *(mg)	Actual GI activity (µmoles/min)
1	AS 1 + E	10	5	100	5	17.0±0.02	2.53
2	AS 1 - E	10	5	-	5	14.26±0.01	
3	AS 2 + E	10	5	100	5	8.82±0.02	3.76
4	AS 2 - E	10	5	-	5	4.75±0.01	
5	AS 3 + E	10	5	100	5	9.70±0.02	8.50
6	AS3- E	10	5	-	5	0.46±0.01	
7	AS 4 + E	10	5	100	5	9.59±0.02	-0.36
8	AS 4 - E	10	5	-	5	9.98±0.01	
9	AS 5 + E	10	5	100	5	4.46±0.01	-0.10
10	AS 5 - E	10	5	-	5	4.57±0.02	
11	AS 6 + E	10	5	100	5	4.05±0.01	-1.53
12	AS 6 - E	10	5	-	5	5.72±0.01	
13	AS 7 + E	10	5	100	5	2.09±0.01	0.95
14	AS 7 - E	10	5	-	5	1.06±0.00	

***± SD of three determinations.**

Table 3. GI activity on different concentrations of enzyme and substrate) and time.

S. No.	Parameters	Amount of fructose produced* (mg)	Actual GI activity (µmoles/min)
I	**Enzyme concentration (mg)**		
	5	0.510 [a]	0.023
	25	1.356 [a]	0.062
	100	3.879 [a]	0.179
	400	10.88 [a]	0.503
II	**Substrate concentration (ml)**		
	10	18.77 [b]	0.82
	5	11.32 [b]	0.47
	2	4.61 [b]	0.16
	1	3.14 [b]	0.10
III	**Reaction Time (min)**		
	0	0.16 [c]	0.89
	30	1.65 [c]	9.16
	60	2.50 [c]	13.87
	90	4.54 [c]	25.19
	120	5.20 [c]	28.86

***Mean of three determinations, [a, b & c] superscripted found significantly different.**

GI activity was studied on 20% pulp extract (Table 4) indicated the reverse reaction (negative direction). This was further confirmed in Table 5 of GI activity when pulp extract was enriched with 10 % glucose and 10 % fructose. The positive value (1.07 µmol/min) of GI was obtained with pulp enrichment by 10% glucose but the negative value (-4.37 µmol/min) of GI was obtained with 10% fructose enriched pulp. In other words, the ratio of fructose: glucose (F/G) was high in pulp extract thus the GI activity was in negative direction. However, addition of glucose (10%) decreases this ratio and the reaction occurred in positive direction. Again, addition of fructose (10%) caused increase of F/G ratio thereby resulting in higher negative activity of GI. Thus, it was confirmed that since the GI activity was bidirectional, the ratio of F/G in jackfruit pulp extract was decisive in the direction of GI activity (Gautam et al., 2010). Shamsuddin et al., 2009 and Li et al., 2017, reported the three fold increased in glucose: fructose during later stage of ripening of jackfruit which reaches to one (0.9 to 1.2). Hence, it was proved that jackfruit pulp was having more of fructose than glucose.

Table 4: GI activity on various concentration of jackfruit pulp extract.

S. No.	Phosphate Buffer (ml)	GI Enzyme (mg)	Substrate in ml (Pulp Extract)	Distilled water (ml)	Amount of fructose produced due to GI activity* (mg)	Actual GI activity (µmoles/mins)
1	10	100	0.0	10	0.61	-
2	10	100	0.2	9.8	0.67	-1.85
3	10	100	0.5	9.5	0.85	-1.68
4	10	100	1.0	9.0	1.14	-1.41
5	10	100	2.0	8.0	1.55	-1.03
6	10	100	5.0	5.0	2.58	-0.08
7	10	00	5.0	5.0	2.06	-

***Mean of three determinations**

Table 5: GI activity on enriched pulp extract of jackfruit with Glucose (10 %) and Fructose (10%).

S.NO.	Samples	Phosphate Buffer (ml)	GI Enzyme (mg)	Substrate (Pulp Extract) (ml)	Distilled water (ml)	Amount of Fructose Produced* (mg)	Actual GI activity (µmoles/mins)
1	ANS 1 + E	10	100	5	5	2.69	1.07
2	ANS 1 – E	10	-	5	5	1.53	
3	ANS 2 + E	10	100	5	5	10.52	-4.37
4	ANS 2 – E	10	-	5	5	15.25	

***Mean of three determinations**

ANS 1 + E ⟶ Enriched pulp (10% glucose) with GI enzyme
ANS 1 – E ⟶ Enriched pulp (10% glucose) without GI enzyme
ANS 1 + E ⟶ Enriched pulp (10% glucose) with GI enzyme
ANS 1 – E ⟶ Enriched pulp (10% glucose) without GI enzyme

Since, GI activity on pulp extract was reported in reverse direction due to the conversion of fructose into glucose because of the higher amount of fructose available in the pulp extract, fructose needed to be removed from the medium for GI to produce more fructose from glucose. Thus, Dowex Monosphere 99Ca/320, an ion resin was used for the separation. Blasé et al., (2008) in U.S. patent 20080044531 also reported the use of Dowex Monosphere 99Ca/320 for the separation of fructose from the sugar containing beverage (fruit juice, vegetable juice and dairy products) to produce sugar-diminished beverage known as low-calorie beverage.

The fractions obtained in present investigation rich in fructose sugar were collected and treated with powdered activated carbon (3% w/v) for 1 hr. at 80°Cto get rid of any odor, color and impurities present (amine odors derived from chromatographic resins). The syrup was formed by evaporating the filtered fractions of enriched fructose at 60°C temperature vacuum evaporator till TSS reaches 60°B. Fennir et al., 2003 concentrated the extracted date sugar syrup

by two methods- conventional heating and microwave heating.

Various fruits have been reported to produce HFS, they include apple, pear, plums, prunes, peaches, nectarine, apricot and grapes (Lapoujade et.al., 2010), date palm(Chaira et al., 2010), Kabkab date fruit(Bahramian et al., 2011) and Parisa et al., 2008. Azevedo & Rodrigues, 2000, obtained high fructose sugar from cashew apple. Date syrup from tamer fruits of two varieties, Birhi and Safari, was prepared on a laboratory scale for replacement of sucrose in pan bread formulations(Sidhu et al., 2003). High fructose Sugar is also reported to obtain from inulin (chicory roots)(Singh et al., 2007) and Jerusalem artichoke (Kim et al., 2006).

CONCLUSION

In present investigation, optimization of GI was done on ripe jackfruit pulp at pH 7.0, temperature 70 ^{0}C time 120 mins on 5 ml substrate with 100 mg of GI. The high fructose content present in pulp and bi-directional property of GI activity caused the reaction in reverse direction hence the fructose was separated first with the help of polystyrene- DVB, gel based strong cationic resin called Dowex Monosphere 99Ca/320. The fractions obtained were used for Glucose-isomerase activity and the glucose was converted to fructose. All the fructose rich fractions were collected and treated with 3% (w/v) of activated carbon and filtered to remove any impurities, color and odor in the solution. The filtrate was then evaporated to 60°B in low temperature (60°C) vacuum evaporator to get fructose rich syrup.

Scope for further studies

The present investigation was to prepare High Fructose Sugar from Jackfruit of Indian variety. Few aspects need further investigation and studies to be conducted as:

1. Establishing standard procedures for commercial extraction of Invert Sugar and HFS from jackfruit.
2. Sucrose replacement with HFS from jackfruit can be studied in fruit juices, preserves, jams, jelly and candies and other bakery products.
3. Stability and Shelf-life study of Invert sugar and High Fructose Sugar should be done.
4. Evaluation of sensory characteristics in combination with various fruit juices.
5. Study of nutritional aspect of Invert Sugar and HFS from jackfruit.

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Abbreviations

HFS:	High Fructose Sugar
GI:	Glucose-isomerase
TSS:	Total Soluble Solid
ml:	mililiter
mg:	miligram
min:	minutes
µmoles:	micromoles
AS:	artificial substrate
E:	enzyme
ANS:	enriched pulp extract
rpm:	rounds per minute
$^{\circ}$C:	degree Celsius
PE:	Pulp extract
hrs:	hours
%;	percentage
g:	gram
$^{\circ}$B:	degree brix

G/F: Glucose to Fructose ratio

Authors' Contribution

Noopur Gautam made an active contribution in designing the experimental work, the main idea of research work, carrying out the experiments in laboratory.

Manoj Kumar has helped in drafting the manuscript.

Shiv Kumar supervised the entire research work guiding and advising time to time and coordinating with all the concern authorities in different organization for smooth working.

Uzma Siddiqui participated in statistical analysis, formatting the manuscript and aligning with the journals' publishing requirements.

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