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Performance evaluation of developed lab scale fermenter

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■ **ABSTRACT :** Processing of raw agricultural produce works as a backbone of agricultural sector. Day by day to fulfill the need of increasing population processed food requires, maintaining quality of raw product as well as to enhance the market rate.This fermentation is done with the breakdown of complex material into the fermented product. Due to such characteristics fermentation helps to make higher growth of agricultural sector in our economy. Fermentation can be done by various ways but basically it may be aerobic and anaerobic kind. In another group it divided as solid state and submerged fermentation. For submerged condition fermentation needs a fermenter. It is a cylindrical vessel with having features like aeration system, agitation unit and monitoring as well as to control parameters of fermentation media. The efficient lab scale fermenter development was started with the selection of vessel body as of stainless steel–316 food grade material because of currently available market fermenter have problems with their glass body, sterilization, construction etc. So as per design consideration with aspect ratio 3:1 and working capacity of 2.0 lit., fermenter body and its assembly parts were developed. Design of fermenter makes feasible to use it as for aerobic as well as anaerobic condition also. The performance evaluation of developed fermenter was carried out on product basis in which the anaerobic fermented product selected as guava cider and in case aerobic it was alpha amylase enzyme. In anaerobic fermentation by using *Sacchromyces cerevisiae* yeast and fermenter, guava pulp kept for fermentation for 36 hrs trials. After centrifugation the cider formed with the features like average pH = 4.17, TSS = 4.7^0Brix and alcohol content = 5.33 per cent. Similarly with the same pulp and proportion fermentation was carried out in glass bottle as a comparative study. In this case also the yield of cider formed in fermenter was observed 10.50 per cent more than in glass bottle. In aerobic condition fermenter model set up was done with aeration unit and with the help of *Bacillus subtilis*, fermentation of chemical media was carried out for 24 hrs at a pH 7. After fermentation time centrifugation was done and crude enzyme collected. By testing its average enzyme activity were observed as 0.22U/ml and compared with standard maltose curve gave significant results as maltose release 0.42 mg. The developed fermenter of 2.0 lit. working capacity have features like multiple use, *in situ* sterilization, rigid structure, simple assembly, easy handling and affordable cost of Rs.27313/-.

■ **KEY WORDS :** Fermentation, Aerobic, Anaerobic, Fermenter, Cider, Amylase

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Agricultural sector is very widely spread to become source of food, employment and money as a backbone of world economy. For the fulfillment of feed requirement of increasing population; agricultural and food processing stream plays vital role in it. In food processing techniques there are many methods are used

for enhance nutritional value of the food and increases the shelf-life of food products. In those techniques fermentation technique is also important and it works effectively. The fermentation method may be aerobic or anaerobic but it results in increasing the market value of that agricultural food processed product (Blakebrough, 1973). Today fermented products in agricultural field plays vital role as a one of the major income source for food processing industries with increase in nutritional and quality parameters. Fermentation technique is important to produce products such as antibiotics, enzymes, amino acids and organic acids. This helps to make food product such as beverages, ethanol and vinegar better than chemically. Byproducts of fermentation are usually chemicals; the cell mass and other major byproducts are highly nutritious and can be used in animal feeds (Benz, 2011).

In fermentation there is need of careful control and monitoring of pH, nutrients, air and agitation.On the basis final product development technique fermentation process mainly differentiated into; batch fermentation, fed batch fermentation,continuous fermentation. On the basis of material system involved, fermentation processes are classified as either: Solid state and submerged culture fermentation Chisti (1999). Anaerobic fermentation is type of fermentation which takes place in the absence of oxygen. Anaerobic fermentation also plays vital role in the alcoholic beverages like wine, cider. In the home scale purpose this fermentation carried out in the glass bottle and for industrial point of view large capacity cylindrical vessels are used. In aerobic fermentation air supply is necessary for the product formation with the agitation. In this method the major role is played by sparger as an aeration unit with controlling and monitoring devices.

The word cider is used for fermented alcoholic beverage made from pressed apple juice as anaerobic fermented product.Cider production is a complex process in which consumption of sugars by yeasts yielding ethanol and CO_2. Cider can be made easily with various fruits like apple, mango, pears, guava etc. A basic classification of cider is into sweet, dry, sparkling, champagne or carbonated beverages Heikefelt (2011). Amylase is the enzymes that break down starch, produced by aerobic fermentation. Amylases can be derived from several sources, such as plants, animals and micro-organisms.In industries the production of amylase from micro-organisms is preferable. The 75 per cent use of industrial produced enzyme mainly in detergents, textiles, starch, baking and animal feed. The level of alpha amylase activity in various human body fluids is of clinical importance e.g. in diabetes, pancreatitis and cancer research (Das *et al.*, 2011).

Fermenter is the heart of fermentation. Fermenters are commonly cylindrical vessels with hemispherical top and bottom, ranging in size from some liter to cubic meters, and are often made of stainless steel and glass. Fermenter is able to provide the optimum environment or condition that will allow supporting the growth of the micro-organisms (Alok and Immanuel, 2014). As fermenter needed to provide all biological, environmental, controlling support to fermentation so there isno universal bioreactor or fermenter. Although there is no universal fermenter, the available fermenter should perform requirements like gentle heat transfer; avoid contamination, easy handling, aseptic in nature, simple in design etc. For a lab scale purpose stirred fermenter vessel has capacity range of 1 lit-50 lit.

The development of multipurpose lab scale fermenter was done because of there are some lacunas in existing lab scale fermenter models in the market are as in its handling, construction, use, design, heat transfer, foaming and higher cost. To meet higher performance from lab scale stirred tank fermenter such as high product yield, antifoaming characteristics, efficient cleaning system and controlling measures, construction, easy assemble and dissemble, easy handling and easy to understand over the available market fermenters, this research work was carried out. Also the major part of research study is deals with comparative study of product like guava cider developed in such developed fermenter and glass bottle fermenter. Similarly performance evaluation of fermenter was carried out on alpha amylase enzyme production in it and compared with standard maltose curve.

METHODOLOGY

Construction details of developed fermenter :

In the development of fermenter is based on design considerations with respect to its aspect ratio *i.e.* 3:1,with this the vessel height and diameter specifications are taken as 300mm and 100mm, respectively and others needed specifications also taken on this basis. By which the total capacity of fermenter is carried out as 2.35 lit.

with working capacity of 2.00 lit. major parts are manufactured from food grade stainless steel Davis (2010) and Jagnani *et al.* (2010).

This developed model having assembly like, from top side D.C. motor supported with flexible clamp fittings on handles of cylindrical vessel closed at its top and bottom side by collars and plates. Further motor shaft is attached to the metal bush by grub screw similarly at another end of bush attached with impeller shaft. Shaft is passes through top head plate from mechanical seal of bearings to avoid the friction loss as well as for balancing. Head plate having two openings serves as pH and temperature sensor insertion point's similarly two extensions on it facilitate external agent addition. Fermenter has major advantage to run fermenter both ways as aerobic and anaerobic, with help of these extensions opening and closing. When one extension pipe on top plate is closed, so another work as exhaust gas outlet for aerobic system. Similarly when one open end used as CO_2 collector by plastic tube connection from extension to the water filled bottle, by keeping air supply closed system serves as anaerobic fermenter. Below the top plate red colored silicon rubber gasket is placed then after it collar is welded to cylindrical vessel. Collar is welded from both ends of vessel. At the bottom side of vessel gaskets are used to avoid leakages from bottom plate, which having connection of drain pipe and valve as a sampling and drain point of model. At bottom side base support is provided to the whole assembly joined by nut-bolts.The two extensions of pipe are welded at the bottom side of vessel which serves as connection point for sterilization unit and another one is a sparger which further connected to air compressor assembly for aerobic condition. Without air supply to sparger works simply a pipe by closing its end by closing cap facilitates as anaerobic condition. The sterilization unit is build up with simple pressure cooker, hydraulic fitting, needle valve, carbon steel pipe and asbestos strip. Heat is given to cooker containing water; the generated steam pass through carbon steel pipe which attached to one extension of fermenter vessel by needle valve simply serves as *in-situ* sterilization unit. The air compressor assembly include air compressor, pressure valve, clamps and tubing's, attached to the sparger.

Materials required for guava cider production:

Guava cider production is done with materials as: Ripen guava, sugar, yeast (*Sacchromyce scerevisiae)* and distilled water.

Lab scale fermenter equipped with assembly for anaerobic fermentation used to formulate the guava cider as low alcoholic beverages. To develop the cider from guava fruit firstly selection of well ripened guava fruit was needed, which was followed with the basic operations like weighing, cleaning, washing and cutting. The enhancement of product yield after the cutting of guava fruit in pieces were blanched for 15-20 min. at about 80^0C temperature. Blanching was helpful to get maximum pulp while pieces of blanched guava grinded by using mixer. The formulated mixer of pulp and seeds separated by using metal sieves, this gave us the pulp of guava fruit as a base source for cider. Then after in that pulp sufficient amount of sugar was added in pulp to get TSS at about 15^0 Brix to get sufficient source of sugar for cider production. Afterwards in 3 per cent yeast solution was made on weight basis with the help of normal hot water and semisolid *Sacchromyce scerevisiae* yeast as a converting agent of sugar into alcohol. Yeast solution and guava pulp mixed properly and fed to the sterilized developed lab scale fermenter model (Fig. 1) by removing top plate of it. Then the whole assembly of fermenter made as for anaerobic condition by making fermenter vessel as close loop system. At sufficient RPM of 350 agitations was provided to enhance the growth rate of yeast as well as to make homogenous mixture and kept this fermentation unit run for the time period ranges from 36 hrs to 72 hrs, at about average temperature of 30^0C up-to getting the constant TSS value of fermented pulp which tested while fermentation process running by using sampling unit and hand refractometer. The online observations like pH, temperature and TSS were recorded for every 6 hrs gap. After 36 hrs of fermentation time TSS readings were found constant. So then after the whole fermented pulp was drained through drain pipe and collected fermented pulp then fed to centrifuge machine for separate out cider and solid particles at a 4000RPM and 20^0C temperature. Formed cider then kept for pasteurization in hot water and with the help of conical flask at a temperature of 65^0C for 15 minutes and then hot filling of cider was done in sterilized bottle. Lastly cider in bottle was kept for storage in refrigerator. This similar process was carried out in three trials, also same pulp and its proportion used in glass bottle fermentation for comparative study.

In which pulp was filled in glass bottle and made it completely airtight by cap having centre drilled point facilitate as connection for plastic tube in water containing bottle for CO_2 collection. The fermentation time were provided as same as lab scale fermenter.

Alpha amylase production :

As per the research guidelines of researchers Vasantha and Hemashenpagam (2012) for the preparation of amylase enzyme with the help of *Bacillus subtilis* from starch following chemicals are required and used in this research.

Chemicals for media preparation :

For volume = 2.0 lit, peptone = 1.2g, $MgSO_4.7H_2O$ =1.0g, starch soluble = 2.0g, KCl= 1.0g and distilled water

The developed fermenter model tested on the product basis as aerobic product alpha amylase enzyme. The fermenter assembly equipped with sparger and air compressor unit used in the aerobic fermentation. The upstream processing followed with chemical media preparation for working capacity of 2 lit by the above listed chemicals. Sterilization of media was carried out in autoclave for 1 hr. and at a120^{0}C temperature and then kept it for cooling. Then media filled in fermenter assembly (Fig. 2) which already sterilized by *in situ* sterilization and then *Bacillus subtilis* added in fermenter vessel through top plate port with the help of micropipette. At the speed of agitation 300 RPM suitable air supply were passed through sparger inserted in fermenter by maintaining the differential pressure through needle valve. The fermentation study carried out for period of 24 hrs, there was requirement of maintaining pH of media at 7 throughout the fermentation. This was made easier by checking online pH values and addition of acid or base as per need to maintain it.

After 24 hrs of fermentation period whole fermented media drain out completely through the drain valve unit. The collected fermented media as a crude enzyme further processed to centrifugation by centrifuge machine to separate out supernatant and pellets at a 5000RPM, 15 minutes and 4^{0}C temperature. Derived supernatant used to test the enzyme activity, for this test procedure as requirement to take one control tube and experimental tube. Then addition of 0.5 ml of 0.05 Molar phosphate solutions in both the tubes and then addition of collected supernatant was done in the experimental tube with the double amount of phosphate solution. Afterwards kept experimental and control tube for incubation in incubator at 40^{0}C temperature for 10 minutes period. To stop the reaction, DNS reagent was added in both the tubes and kept them for water bath for 5 minutes. Finally to check absorbance both tube samples were fed to spectrophotometer at 540 nm wavelength. Experimental tubes were taken in 3 numbers of quantities.

■ RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Performance evaluation of developed fermenter model :

The performance evaluation was carried on product basis as production of two fermented products as guava cider and amylase enzyme in the developed fermenter model.

Guava cider :

With materials like guava fruits, yeast, sugar and distilled water and lab scale fermenter, glass bottle fermenter cider production was carried out in trial and error base method *i.e*. in three trials. Processing and fermentation was carried out in as possible as in hygiene condition, with the all available accessories (Fig.1).

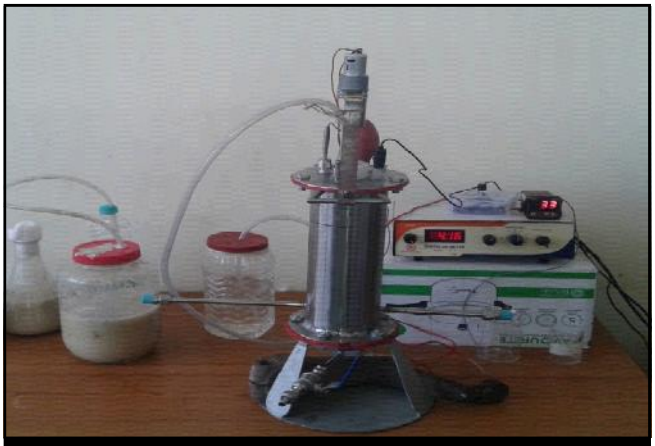

Fig. 1 : Developed lab scale ferementer- anaerobic condition and glass bottle fermenter

The observations were taken in the fermentation period for the gap of 6 hrs for all three trials in developed fermenter. The recorded readings in three trials showed

the directly proportional relationship between pH, TSS and temperature. The minimum TSS of pulp was found in trial II as 4^0 Brix for pH value of 4.16. Agitation helps to improve the fermentation time (Table 1).

The formed cider was tested for analyzing its different contents, such as alcohol was found by alcoholmeter, viscosity by viscometer, acidity by chemical titration, pH, TSS, density (Table 2).

The analysis was done on developed guava cider for three trials. The parameter of average pH=4.17 was measured online as it an integral part of the fermenter assembly (Table 2). TSS measured by using Hand refractometer which was an average 4.7^0 Brix. Acidity of cider was calculated by using chemical titration method, as concern with the available cider in market acidity of developed cider 0.47 per cent satisfactory one and significant to the acidity of guava cider developed by ICAR as 0.42 per cent at TSS 13^0 Brix and alcohol content 4 per cent. The Brookfilled Viscometer was used to identify the viscosity of developed cider with the use of spindle unit. The average density of cider was recorded 0.88 g/ml which was numerically below value of 1 g/ml, also satisfactory. The major important factor as alcohol content of cider was measured by using alcoholmeter available directly in market.

Comparative study of cider :

The guava cider developed in efficient lab scale fermenter model having somewhat yellowish colour. This anaerobic fermented product cider was made side by side also in glass bottle. The provision of glass bottle because of there was lack of availability of lab scale anaerobic fermenter. So selected Borosilicate glass bottle used as fermentation vessel which fed same concentration of pulp as in developed lab scale stainless steel fermenter. Glass bottle provided with leak proof outlet for CO_2, in water filled another plastic bottle. The fermentation period provided to this glass bottle was as equal to the developed fermenter. In case of glass bottle there was no provision of any online measurement system and agitation unit. After completion of fermentation period, with the same downstream processing as used in case fermenter vessel cider gets formulated and stored. This kind of system majorly used in home scale purpose. By providing similar fermentation time to glass bottle fermenter as per the time required for developed fermenter to meet the constant TSS of pulp *i.e.* 36 hrs. The analysis was carried out on the developed cider through glass bottle fermentation in the three trials for the different parameters (Table 3).

The comparison was undertaken between cider,

Table 1 : Online observations in lab scale developed fermenter for guava cidertrials –I, II and III

Trial No.	I			II			III		
Sr. No	pH	TSS ^{0}Brix	Temp. ^{0}C	pH	TSS ^{0}Brix	Temp. ^{0}C	pH	TSS ^{0}Brix	Temp. ^{0}C
1.	4.31	15	34	4.6	15	33	4.33	15	34
2.	4.31	10.5	28	4.13	7.0	31	4.22	5.0	31
3.	4.33	8.5	27	4.19	5.0	30	4.18	5.0	30
4.	4.33	7.0	27	4.15	4.0	32	4.17	5.0	32
5.	4.34	6.0	28	4.16	4.0	32	4.14	5.0	31
6.	4.32	5.0	28	4.17	4.0	33	4.11	5.0	32
7.	4.29	5.0	29	4.16	4.0	32	4.11	5.0	32

Table 2 : Guava cider analysis produced by developed fermenter

Parameters → / Trial No. ↓	pH	TSS ,^{0}Brix	Acidity,%	Viscosity, MPa.s	Density, g/ml	Alcohol,%
I	4.5	5	0.48	56.1	0.94	5
II	4.02	4	0.45	56.8	0.85	7
III	4.00	5	0.50	55.5	0.85	4
Average	4.17	4.77	0.47	56.13	0.88	5.33
Mean	4.16	4.66	0.47	56.13	0.87	5.19
S. D	0.28	0.57	0.025	0.65	0.051	1.52

developed fermenter and glass bottle. The analysis parameters does not shows any major significant change in between them; but as concern with products yield point the beneficial characteristics of developed fermenter. In the form of percentile the average yield of cider formulated in fermenter vessel was 10.50 per cent more than that of cider developed through bottle fermentation. This seems to be enhancing product yield due to the provision of agitation assembly in efficient lab scale fermenter. In case of easiness and sampling facility also developed model is better than that of glass bottle for anaerobic condition (Table 4).

Alpha amylase enzyme :

Amylase enzyme was produced by using chemicals and developed fermenter, after developing testing of crude enzyme were followed. The Table 5 shows the values of optical density and respective enzyme activity shown by spectrophotometer in concern with experimental tubes which undertaken in trial I and II.

Fig. 2 : Lab scale aerobic stirred fermenter

Table 3 : Guava cider analysis produced by glass bottle fermenter

Parameters → / Trial No. ↓	pH	TSS ,^{0}Brix	Acidity,%	Viscosity, MPa.s	Density, g/ml	Alcohol,%
I	3.8	5	0.51	53.4	0.92	4
II	4.06	4	0.53	54.8	0.9	6
III	3.9	4	0.54	53.4	0.89	5
Average	3.92	4.33	0.53	53.87	0.90	5.00
Mean	3.92	4.31	0.53	53.86	0.90	4.93
S. D.	0.13	0.58	0.02	0.81	0.02	1.00

Table 4 : Comparative results of guava cider

Parameters assembly → / ↓	pH	TSS ,^{0}Brix	Acidity,%	Viscosity, MPa.s	Density, g/ml	Alcohol,%	Avg. yield, ml /100 ml
Cider (Fermenter)	4.17±0.28	4.7±0.57	0.47±0.025	56.13± 0.65	0.88±0.051	5.33±1.52	74.33
Cider (Bottle)	3.9±0.13	4.33±0.57	0.52±0.015	53.7±0.80	0.90±0.015	5.00±1	67.33

Table 5: Analysis of amylase enzyme activity

Trial No.	Experimental tubes	Optical density	Enzyme activity, U/ml
	T_1	0.10	0.20
I	T_2	0.12	0.24
	T_3	0.12	0.24
	Average	0.11±0.01	0.23±0.02
	T_1	0.17	0.17
II	T_2	0.28	0.28
	T_3	0.22	0.22
	Average	0.22±0.05	0.22±0.05

The volume of enzyme used for experimental tubes 0.5 ml and 1 ml, respectively to trial I and II

As enzyme activity data shows positive response to spectrophotometer analysis the results were satisfactory. The average enzyme activity was found in aerobic fermentation study in developed fermenter model equals to 0.22 U/ml.

Comparative study of alpha amylase enzyme :

To check the enzyme activity, the comparison was carried out with the standard maltose curve. On the standard maltose curve the average values of trials of enzyme activity found in fermented product which developed in lab scale fermenter were compared; this one as a standard protocol. Maltose curve was generated by using standard maltose procedure for standard maltose solutions like 0ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml. After finding the optical densities of each standard solution by using spectrophotometer, graph plotted between concentration of maltose against the respective standard solution and absorbance shown by spectrophotometer for respective standard maltose solution.

This standard maltose curve satisfactorily notifies that the absorbance measured at respective standard maltose solution gave slightly linear relationship with the maltose concentration. The average absorbance was recorded as 0.22 nm, against this reading by maltose curve concentration of maltose was found 0.42 mg. The study done by Vasantha and Hemashenpagam (2012) on the amylase production by *Bacillus subtilis* with the same chemical media shows that concentration of maltose after 24 hrs fermentation as 0 mg. Against their standard fermentation results the observed readings in this research work in developed lab scale fermenter were high maltose concentration and significant also (Fig. 3).

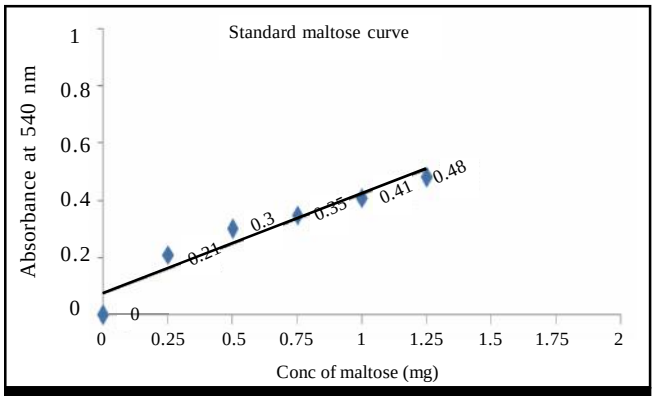

Fig. 3 : Standard maltose curve

Conclusion :

The development of stirred tank fermenter with standard considerations found effective in SS-316 body for the working capacity of 2.00 lit for both aerobic and anaerobic fermented products. This lab scale fermenter facilitates functions like online pH-temperature monitoring, *in-situ* sterilization, easy handling, rigid structure, multiple use, simple design etc. Guava cider production as anaerobic fermented product is feasible in this lab scale fermenter, due to agitation by speed of fermentation increased and foam formation reduced better than glass bottle fermenter. The average yield of cider was observed in process of lab scale fermenter more than 10.50 per cent that of glass bottle fermentation. Amylase enzyme production in lab scale fermenter also gives satisfactory results. The maltose release by produced enzyme against the standard maltose curve was found significant as 0.42 mg. Overall this lab scale fermenter works effectively in both methods of fermentation.

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