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Apple Pomace: A Potential Substrate for Ethanol Production

[1]Pranita A. Gulhane*, [2]Ashok V. Gomashe, [3]Kalyani Kadu
Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS), India
*pranita12 a gmail.com

Abstract: *Apple pomace is the main by-product resulting from pressing apples for juice or cider. Apple pomace serves as the potential source of ethanol. Apple pomace is a waste and its disposal is a major environmental problem, but being a precious resource, its utilization is a challenge and opportunity to the scientists and technologists. In the present study waste apple pomace left after juice extraction which is generally dumped is used as a substrate for ethanol production in solid state fermentation (SSF) system. Ethanol production was carried out by using three different fungi Saccharomyces cerevisiae, Fusarium oxysporum and Aspergillus foetidus. Percentage of Ethanol estimated by iodometric titration method was found to be highest (1.3702 gm%) by using a combination of three fungi Saccharomyces cerevisiae, Aspergillus foetidus and Fusarium oxysporum followed by Saccharomyces cerevisiae (1.326 gm%) and that of combination of Aspergillus foetidus and Fusarium oxysporum (1.292% gm%). Research on the conversion of fruit waste into ethanol will contribute substantially to bio-based economy.*

Keywords: *Apple Pomace, Ethanol, Saccharomyces cerevisiae, Fusarium oxysporum, Aspergillus foetidus*

1. Introduction

India has a diverse agro-climatic condition that has enabled the country to produce a wide variety of horticultural crops. Amongst these crops, apple (*Malus pumila* occupies a prominent position in India and world [1]. Apple processing industries are one of the major industries of Himachal Pradesh, Jammu and Kashmir, and Uttaranchal in India, manufacturing various products like juice, concentrates, wine, cider, canned slices, etc. Apple pomace is a left over residue after juice extraction containing peel, seeds and remaining solid parts and represents about 25-35% of the weight of the fresh apple processed [2][3].

Biofuel production is rapidly growing as the world encounters pollution problems due to burning of petroleum and coal based fuels. This reinforces the fact that alternative fuels are important from both environmental and energy security point of view. Apple pomace is the residue left after juice extraction and constitutes about 25-35% of the weight of fresh fruit [4] [5]. It contains a large amount of water and sugar, a small amount of protein, and has a low pH. More than 500 food processing plants in the United States produce a total of about 1.3 million metric tons of apple pomace per years. The direct disposal of agro-industrial residues as a waste in the environment represents an important loss of biomass, which could be bioconverted into different metabolites, with a higher commercial value.

Apple pomace is a rich source of pectin besides other nutrients like carbohydrates, dietary fibers, minerals and vitamin C [6] [7]. Thus, it can be good substrate for the fermentation, pectin esterase enzyme production or to make animal feed, citric acid, ethanol, and bio-color production [8] which otherwise require a costlier medium for their production [9].

Because of the increasing demand for ethanol, alternative and non-conventional raw materials are under research [10] [11]. Some researchers have documented the potential for ethanol production from fresh wet pomace and this represents a 20% of energy recovery from the total energy in pomace [12]. The present study investigated for the bio-conversion potential of apple pomace to produce bioethanol. Apple pomace is a waste and its disposal is a major environmental problem, but being a precious resource, its utilization is a challenge and opportunity to the scientists and technologists. In the present study waste apple pomace left after juice extraction which is generally dumped is used as a substrate for ethanol production in solid state fermentation (SSF) system.

2. Methods

2.1. Culture Collection

The following fungal cultures such as *Saccharomyces cerevisiae* (NCIM-3189), *Fusarium oxysporum* (NCIM-1043) and *Aspergillus foetidus* (NCIM-514) were collected from National Chemical Laboratory (NCL), Pune. Where, NCIM= National Collection of Industrial Microorganisms.

2.2. Raw Material Used

Apple pomace was collected from fruit juice vendors in Nagpur city in sterile polythene bag and immediately transferred to the microbiology laboratory for further experimentation.

2.3. Solid State Fermentation

At the first step in experiment three sterilized conical flasks of 500ml capacity were taken and in each flask 50gm of apple pomace was added. Each flask was labeled as per the fungal culture added. In one flask only *Saccharomyces cerevisiae* was added, in second flask *Aspergillus foetidus* and *Fusarium oxysporum* were added while in third flask a combination of three fungi i.e. *Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum* were added to the flask containing apple pomace. In each flask ammonium sulphate (1.8%) i.e. 1.3gm and potassium bisulphate (300ppm) was added as supportive material of fungi. The flasks were sealed air-tight to maintain anaerobic condition and kept in stable state for undergoing solid state fermentation for 4-5days [13].

2.4. Iodometric Titration Method

After fermentation the inoculum in each flask was pressed by using sterilized muslin cloth. Brown coloured extracts were obtained which were collected in different beakers and distributed in test tubes. By iodometric method the percentage of ethanol contained in each flask was estimated.

In a clean dry test tube 1ml of distillate was taken (extract) from each flask and 5ml of K_2Cr_2O- was added. Now, it was kept in ice bath for 5 min. Then concentrated H_2SO_4 was added slowly with continuous stirring. The flask was then kept in boiling water bath for 5-7 min. All the flasks were allowed to cool and the contents were transferred into other respective flasks, each of which contained 1 gm of potassium iodide. Each flask was then washed with distilled water and washing was added in flask and mixed well. The flask was kept stable for 5 min.

Now, in each of these flasks 1-2 drops of starch indicator was added. The burette was filled with 0.05N thiosulphate and it was used for titration. During titration, firstly colour becomes brown then the titration was continued till the colour of the solution of each flask get changed to pale yellow. Titration was repeated for three times for each flask and the observations were noted. The percentage of ethanol was calculated by using following formula $N_1V_1 = N_2V_2$.

Therefore $V_2 = N_1 V_1 / N_2$

$$= \frac{\text{Normality of hypo} \times \text{Volume of hypo}}{\text{Normality of dichromate solution}}$$

Where, N_1 = 0.05 N

V_1 = Burette reading

N_2 = 0.3N i.e. normality of dichromate

Let, the amount of dichromate utilized= y ml

Therefore, $y = 5 - x$

Gram % of alcohol = y X alcohol factor X 100

Thus, on the basis of the value of V_2 obtained, the gm% of Ethanol was determined for each combination of fungi along with the apple pomace.

3. Result and Discussion

The present study investigated the bio-conversion potential of apple pomace to produce ethanol. Apple pomace left after juice extraction was collected from fruit juice vendors in Nagpur city. The

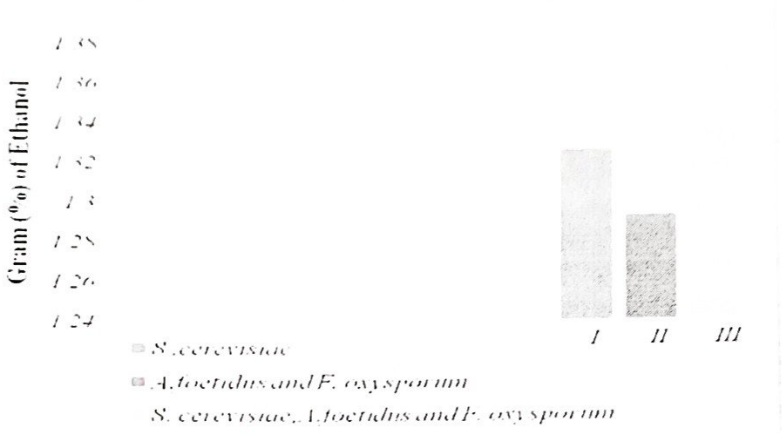
three different types of fungi such as *Saccharomyces cerevisiae*, a combination of two fungi *Aspergillus foetidus* and *Fusarium oxysporum* and a combination of three fungi *Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum* were used for the study.

Iodometric method was carried out for the estimation of ethanol. After fermentation the extract of each combination of *Aspergillus foetidus* and *Fusarium oxysporum* flask was treated with thiosulphate solution till the colour get changed to pale yellow. From these readings the percentage of ethanol was estimated using the given formula. It was found that a combination of *Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum* has given highest percentage of ethanol (1.3702 gm%) followed by *Saccharomyces cerevisiae* (1.326 gm%) and that of combination of *Aspergillus foetidus* and *Fusarium oxysporum* (1.292%) (Table 1) (Graph 1).

Table1. *Ethanol Production from Apple Pomace*

Fungi Used	Ethanol (gm%)
Saccharomyces cerevisiae	1.326 gm%
Aspergillus foetidus and *Fusarium oxysporum*	1.292 gm%
Saccharomyces cerevisiae, *Aspergillus foetidus* and *Fusarium oxysporum*	1.3702 gm%

It was found that the combination of two and three fungi have given more percentage of ethanol than that of the fungi alone. The dried apple pomace was then used as an animal feed. An overview of the present project showed that solid state fermentation of apple pomace has a large economic potential for conversion into ethanol. Solid state fermentation of apple pomace is a simple, high yielding and economically feasible process.



Graph1. *Gram (%) of Ethanol Production from fungi*

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These results were correlated with that of the previous findings in which the researchers used different fungi for the production of ethanol which included the *Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum* [13]. In some studies, solid state fermentation had performed for the effective production of ethanol [14], the same process was performed in the present study. Thus in future solid state fermentation could become a potential tool for solid waste management of food processing plant to prevent environment pollution as well. The present study has shown a promising potential for utilizing apple pomace as a noble substrate for the production of ethanol. This was proved in the optimization study of ethanol production from apple pomace [15]. Thus, apple pomace has a large economic potential for conversion into ethanol.

4. Conclusion

The present study was conducted for ethanol production from apple pomace by using three different types of fungi. It was concluded that ethanol was produced successfully from all three different fungi and their combination. The highest percentage of ethanol was produced by the combination of

Saccharomyces cerevisiae, *Aspergillus foetidus* and *Fusarium oxysporum* has given highest production (1.3702 gm %) while *S. cerevisiae* alone has given (1.326 gm %). However, a combination *Aspergillus foetidus* and *Fusarium oxysporum* have given (1.292 gm %) of ethanol.

Research on the conversion of fruit waste into ethanol will contribute substantially to bio-based economy. The future goal of this research should be focused on investigation of the feasibility of utilizing fruit waste into ethanol through simple method. Utilization of lipid and carbohydrates obtained from food waste for ethanol production is an excellent example to demonstrate the enormous potential of waste valorization for building a sustainable society.

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Authors' Biography

Dr. Pranita has completed her Master of Science in Microbiology as well as Ph.D. on Hospital Airborne Pathogens from S.G.B. Amravati University (MS). She has obtained a major research project under Women Scientist Scheme-A from Department of Science and Technology, New Delhi. Her research was on 'Mutilated Currency Notes as a Source of Human Infection'. She is presently working as an Ad-hoc Lecturer in Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS). She has received 'A Promising Young Scientist Award in Life Sciences'. Her participation in National and International Conferences and paper presentation is noteworthy. She has published research papers in National and International Journals having good impact factor.

Dr. Ashok Gomashe is presently working as an Associate Professor in Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS). He has thirty three years of teaching experience. He has published seventy four research papers in National and International Journals having good impact factor. He has presented papers in National and International Conferences. He is Ex-Registrar of R.T.M. Nagpur University, Nagpur. He is Ex-Chairman of B.O.S. of Microbiology, Ex-Dean Faculty of Science, Ex- Management Councillor, R.T.M. Nagpur University. He is recognized Guide for the Ph.D. students. He has received 'Ideal Teacher Award', 'Adarsh Shikshak Puraskar', 'Best Teacher Award' and 'Environment Protection award'. He is solicited as 'World Scientist' by the Government Information Reliable Source of Information Data, Poland. He is recognized as 'Ruby of Indian Microbiology'.

Ms. Kalyani has completed her Bachelor of Science in Microbiology. She was the Post Graduate student in Department of Microbiology. She has completed Master of Science in Microbiology from Department of Microbiology, S.S.E.S.A's Science College, Nagpur (MS).