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PRODUCTION OF BIOETHANOL FROM *CITRUS LIMETTA* AND *CITRUS MAXIMA* FRUIT WASTE BY BATCH FERMENTATION

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ABSTRACT

In the current study an attempt was made to standardize various parameters to increase the production of bioethanol from fruit pulp and peel waste. *Citrus limetta* pulp, *Citrus maxima* pulp and peel were used. Hot water pretreatment was found to be best suitable than that of dilute acid pretreatment. pH 3 was found to be more effective for fermentation reactions. Two different enzymes pectinase and α-amylase was used in combination with three different organisms, namely *Saccharomyces cerevisiae, Kluyveromyces marixianus* and *Zymomonas mobilis* at room temperature and 50°C. In *Citrus limetta* pulp and *Citrus maxima* pulp and peel the highest ethanol yield (24.61%) was obtained at pH 3 using Pectinase enzyme and *Kluyveromyces marixianus* maintained at 50^0C.

KEYWORDS: **Bioethanol, *Citrus limetta, Citrus maxima*, batch fermentation, pulp waste, peel waste**

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INTRODUCTION

Bioethanol as an alternate source of energy has received special attention worldwide due to depletion of fossil fuel. Moreover, the principle fuel used as a petrol substitute for road transport vehicles is bioethanol. Ethanol or ethyl alcohol (C_2H_5OH) is a clear, colourless, flammable, oxygenated hydrocarbon, biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. . It also has a number of advantages over conventional fuels. It comes from a renewable resource. Ethanol has a higher octane (ability to resist compression) rating than gasoline and petrol, enabling combustion engines to run at a higher compression ratio and thus giving a superior net performance[1 & 2]. Ethanol's flammability in air is lower than that of gasoline, which reduces the number and severity of vehicle fires. Also the vapour pressure of alcohol is greater and the heat of vaporization is higher than that of gasoline, which is primarily responsible for the increased power outputs using alcohol. It reduces harmful exhaust emissions such as carbon monoxide and hydrocarbons. Ethanol can be produced from biomass by the hydrolysis and sugar fermentation processes. Biomass wastes contains a complex carbohydrate polymers such as cellulose, hemi cellulose and lignin. The process of conversion of complex carbohydrates to simple sugars is usually carried out by pretreating the biomass with acids or enzymes. The hydrolysed sugars are then converted to alcohol by fermentation. The lignin which is also present in the biomass is normally used as a fuel for the ethanol production plants boilers. There are three principle methods of extracting sugars from biomass. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis. Biochemically ethanol is a 2 carbon alcohol, which can be obtained from the fermentation of carbohydrates. Ethanol sources may include sugars, starch and cellulose. Micro-organisms can produce ethanol by the process of fermentation only from reducing sugars. This process has been exploited by mankind since time immemorial for the production of alcoholic beverages from various plant materials. For the production of fuel ethanol plant sources like sugarcane and corn starch has been used extensively by the United States of America and Brazil[3]. But these sources are also used as staple food in many parts of the globe and hence there exists a very fine line between food source and fuel source. To overcome this problem, we need to use plant material, preferably non edible sources. But again a problem arises for the mass plantations of such crops. Since the cost of raw materials can be as high as 40% of the bioethanol cost[4], recent efforts have concentrated on utilizing lignocellulose[5]. With urbanization and encroachment in natural environment availability of agricultural land for cultivation is also a problem. Research efforts are focused to design and improve a process, which would produce a sustainable transportation fuel using low cost feed stocks. Many agricultural raw materials rich in fermentable carbohydrates were tested worldwide for bio-conversion from sugar to alcohol, but the cost of carbohydrate raw materials has become a limiting factor for large scale production by the industries employing fermentation processes[6]. To achieve significant economic and environmental benefit large amount of food wastes can be utilized to produce ethanol. Utilization of fruit waste for bioethanol production is one of the best options to reutilize fruit waste. One example of raw material is pineapple waste that is converted to bioethanol[7]. The wastes contain valuable components such as sucrose, glucose, fructose and other nutrients[8]. Lignocellulose is the major structural component of woody plants and non woody plants. The use of mango peel as a source of pectin and fibre production also has been reported[9]. Previously ethanol production from orange peel was reported[10,11,12]. Ethanol productions from banana and pineapple peels were also investigated[13 & 14]. Dried orange peels have a high content of pectin, cellulose and hemi cellulose, which make it suitable as a fermentation substrate when hydrolyzed. Insoluble carbohydrates are present in the cell walls of the peels, particularly in the form of

pectin, cellulose and hemicellulose. Various varieties of microorganisms may be used for the fermentation of bio-ethanol from reducing sugars. Yeast (*Saccharomyces cerevisiae)* has been used as major ethanol producing micro-organism[15]. Alcoholic fermentation, which consists of saccharification by invertase enzyme was also studied previously[2]. This is followed by enzymatic fermentation catalysed by zymase produced by *Saccharomyces cerevisiae.* The content is then fermented for ethanol production. Another wild yeast named *Kluyveromyces marixianus* appears to be particularly promising for the production of bio-ethanol. *Zymomonas mobilis,* a gram negative anaerobic bacterium is another suitable organism for ethanol production. *Zymomonas mobilis* has been used in tropical areas to make alcoholic beverages from plant sap[16]. In the present study fruit wastes of *Citrus limetta* and *Citrus maxima* was used. Both the fruits belong to Rutaceae and used for its juice. After extraction of juice more than 60% -70% of the pulp becomes a source of fruit pulp waste. The study was carried out with an aim to use these fruit wastes and produce bioethanol using fermentation technology.

MATERIALS & METHODS

Plant material

Fresh ground waste pulp of *Citrus limetta* was collected from local juice shops after juice extraction and was used as one of the plant sources. *Citrus maxima* fruits were collected directly from the trees. The fruit pulp was separated from the peel. The pulp was crushed in a blender to extract the juice. The de-juiced waste pulp was used as the second plant source. The separated peel of *Citrus maxima* was used as the third plant source. The initial moisture content of the three samples was determined. Further, 25g of each of the samples was weighed and transferred to five sterilized culture bottles. 100 ml of hot boiled water was added to each of the bottles. The pH was adjusted and two sets were maintained, for both pH3 and pH4. These substrates were then subjected to pre-treatment.

Pre-treatment

Samples were subjected to dilute acid pre-treatment (DAP) and hot water pre-treatment (HWP) The dilute acid pre-treatment was carried out using dilute sulphuric acid (0.1N). The samples were soaked in the dilute acid overnight and used for further analysis. For hot water pre-treatment, samples were autoclaved at 121^0C at 15 psi pressure for 30 minutes. It was then cooled to room temperature and used for further analysis.

Saccharification

Enzymatic saccharification was carried out using α-amylase and pectinase separately. 0.50 gm of commercial pectinase and 0.12 gm of α-amylase were added into the respective bottles. The bottles were incubated at room temperature (25^0C) for a period of 48 hours.

Fermentation

After enzymatic saccharification, the three different microorganisms were introduced to the pre-treated and saccharified samples. Out of the five bottles used for each set, Saccharomyces *cerevisiae, Kluyveromyces marixianus* (MTCC 4136) in two bottles and *Zymomonas mobilis* (MTCC 2427) was added respectively in each bottle. Fifth bottle without any organisms was maintained as control. Replicates were maintained for all the treatments. The culture bottles inoculated with the *Saccharomyces cerevisiae, Kluyveromyces marixianus* (MTCC 4136) and *Zymomonas mobilis* (MTCC 2427) cultures were then allowed to ferment at room temperature. One set of bottle inoculated with *Kluyveromyces marixianus* (MTCC 4136) culture was stored at 50^0C in an incubator. A control bottle without any culture was also maintained at room temperature (25^0C). The samples were allowed to ferment for 10 days.

Ethanol Recovery

After 10 days of incubation the samples were filtered aseptically using a filter paper supported on a funnel. This ensured the stopping of fermentation by separating the fermented ethanol from the cellular biomass and the

substrate (saccharified lignocellulose). The filtrate was then distilled to obtain pure ethanol.

Content Analysis

Two main tests were conducted under content analysis of the samples at various stages of fermentation experiment. The reducing sugar content was measured using the dinitro salicylic acid test (DNS test) and the percentage of ethanol was determined by potassium dichromate and potassium iodide titration method. Reducing sugar content of the three samples was analysed before pre-treatment, after pre-treatment, after enzyme saccharification and after fermentation. Ethanol percentage was estimated initially before pre-treatment and after the fermentation process.

Ethanol amount was estimated by using the following formula:
Moles (dichromate) to oxidize alcohol = moles added – moles spent by sodium thiosulphate
Volume of alcohol in essay = 58.6 × Moles (alcohol)

RESULTS

In the current study, attempts were made to standardize the various parameters such as pH, pretreatment of material, concentrations of various enzymes and the micro-organisms required for the successful conversion of fruit wastes of *Citrus limetta* and *Citrus maxima* into ethanol.

Pretreatment

Pretreatment is done for all plant sources to enhance hydrolysis of complex carbohydrates which enables the micro-organisms to readily act upon. Two types of pretreatments were carried out namely hot water pretreatment and dilute H_2SO_4 treatment, among which hot water pretreatment was found to be better.

Table1
Pre-treatment of fruit pulp and peel

Types of pretreatment	Content of reducing sugar (mg/ml)
Hot water pretreatment	3.1
Dilute acid pretreatment	1.0

pH

pH plays a very important role in any chemical conversion. An attempt was made to study the effect of pH on the able conversion of complex carbohydrates from fruit waste into bioethanol by using various enzymes like pectinase and α-amylase along with three different micro-organisms. It was showed, that pH3 was found to be more effective in the production of bioethanol from fruit wastes. The quantity of bioethanol yield at pH 3 was significantly higher than at pH 4.

Citrus limetta pulp

Reducing sugar analysis

Citrus limetta pulp when maintained at pH3, there was a gradual increase in reducing sugar following pretreatment and further increased when treated with pectinase, but in pH4 it was recorded that reducing sugars showed a narrow decrease following pretreatment, but showed an increase in reducing sugar levels following pectinase treatment. In both the pH there was a decrease in reducing levels following fermentation in all the organisms and at all the temperatures maintained. This shows the efficiency of organisms in converting the sugar to ethanol. In the reactions containing α-amylase also, the scenario was same. The pH 3 & 4 both sowed increase in reducing sugar levels following enzyme treatments and reduce in sugar levels following fermentation. (Table 2 & 3).

Table 2
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After Pre-treatment	After Pectinase treatment	After Fermentation (mg)	Initial (mg)	After Pre treatment	After Pectinase Treatment	After Fermentation (mg)
Saccharomyces cerevisiae (25°C)	0.09	0.94	4.7	0.38	0.45	0.28	2.2	0.23
Kluyveromyces marixianus (25°C)	0.07	0.18	3.5	0.23	0.42	0.20	2.9	0.22
Kluyveromyces marixianus (50°C)	0.08	0.21	3.4	0.25	0.52	0.19	3.4	0.37
Zymomonas mobilis (25°C)	0.09	0.16	7.2	0.25	0.49	0.20	4.4	0.37
Control	0.10	0.23	4.5	0.45	0.51	0.24	4.7	0.33

Table 3
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After Pre-treatment	After α-amylase treatment	After Fermentation (mg)	Initial (mg)	After Pre-treatment	After α-amylase treatment	After Fermentation (mg)
Saccharomyces cerevisiae (25°C)	0.06	0.10	4.3.	0.11	0.10	0.42	3.8	0.10
Kluyveromyces marixianus (25°C)	0.08	0.09	4.1	0.09	0.07	0.43	3.2	0.15
Kluyveromyces marixianus (50°C)	0.09	0.09	3.3	0.15	0.07	0.46	2.7	0.20
Zymomonas mobilis (25°C)	0.07	0.07	5.0	0.15	0.04	0.45	4.2	0.14
Control	0.06	0.10	4.8	0.14	0.03	0.45	3.9	0.15

Ethanol percentage

Citrus limetta pulp showed the presence of 1.62% of ethanol initially. In the reaction containing pectinase, there was a significant increase in the percentage of ethanol produced following fermentation reaction. pH 3 recorded the highest ethanol yield. The highest percentage of ethanol yield was recorded at 15.38 % when fermentation was set at 50°C at pH 3 when fermented using *Kluyveromyces marixianus.* The reaction containing *Kluyveromyces marixianus* and pectinase that left at room temperature (25°C) showed only 11.69 % of bioethanol production. At pH 4 again, the same combination at 50°C was showing the highest yield of 14.65 %, which may be due to the activity of *Kluyveromyces marixianus* is maximum at 50°C than at room temperature (25°C). But when α-amylase was used as enzyme source, the same combination at 50^0C recorded the lowest percentage (6.29 %). In the reactions with only pectinase and no organism, only lowest yield of 8.7 % was recorded (Table 4 & 5).

Table 4
Ethanol percentage Estimation - Citrus limetta pulp

Pectinase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25˚C)	1.62%	10.28%	1.62%	9.81%
Kluyveromyces marixianus (25˚C)		12.04%		10.25%
Kluyveromyces marixianus (50˚C)		15.38%		14.65%
Zymomonas mobilis (25˚C)		11.69%		9.22%
Control		8.7%		8.7%

Table 5
Ethanol percentage Estimation - Citrus limetta pulp

α-amylase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25˚C)	1.62%	8.49%	1.62%	8.64%
Kluyveromyces marixianus (25˚C)		8.05%		7.32%
Kluyveromyces marixianus (50˚C)		6.29%		6.59%
Zymomonas mobilis (25˚C)		8.7%		8.05%
Control		6.59%		8.49%

Citrus maxima pulp

Reducing sugar analysis

In *Citrus maxima* pulp, following the hot water pretreatment, there was an increase in reducing sugars at both the pH. With both pectinase and α-amylase, there was an increase in reducing sugar and decrease in reducing sugars after fermentation with all the microorganisms used in the study irrespective of the temperature, at both pH 3 and pH 4 (Table 6&7).

Table 6
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After Pre-treatment	After pectinase treatment	After Fermentation (mg)	Initial (mg)	After Pre-treatment	After Pectinase treatment	After Fermentation (mg)
Saccharomyces cerevisiae (25˚C)	0.21	0.73	3.7	0.15	0.16	0.62	2.8	0.19
Kluyveromyces marixianus (25˚C)	0.20	0.69	3.1	0.18	0.26	0.66	2.8	0.16
Kluyveromyces marixianus (50˚C)	0.20	0.71	2.7	0.15	0.20	0.64	2.8	0.16
Zymomonas mobilis (25˚C)	0.16	0.74	3.5	0.12	0.21	0.61	1.8	0.18
Control	0.19	0.73	2.2	0.11	0.20	0.68	2.3	0.17

Table 7
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After Pre-treatment	After α-Amylase treatment	After Fermentation (mg)	Initial (mg)	After Pre-treatment	After α-Amylase treatment	After Fermentation (mg)
Saccharomyces cerevisiae (25°C)	0.26	0.70	2.8	0.14	0.26	0.69	1.3	0.19
Kluyveromyces marixianus (25°C)	0.23	0.71	2.8	0.17	0.23	0.71	1.3	0.20
Kluyveromyces marixianus (50°C)	0.20	0.65	2.8	0.13	0.25	0.64	1.7	0.18
Zymomonas mobilis (25°C)	0.21	0.72	1.8	0.13	0.22	0.66	1.2	0.19
Control	0.26	0.69	2.3	0.14	0.22	0.65	1.8	0.18

Ethanol Yield

Citrus maxima pulp recorded an initial ethanol concentration of 0.22 % at pH 3 and pH 4. In the reactions containing pectinase and α-amylase, there was a significant increase in ethanol percentage. The highest yield of ethanol was recorded at pH 3. The highest percentage of ethanol yield at 19.33 % was seen when fermentation was carried out using pectinase enzyme and *Kluyveromyces marixianus* maintained at 50°C at pH 3. The combination of *Saccharomyces cerevisiae* with pectinase enzyme at room temperature (25°C) yielded 19.04 % of bioethanol. The lowest bioethanol yield (8.93 %) was obtained in the reactions containing pectinase and *Saccharomyces cerevisiae* at pH 4. In the fermentation samples containing α-amylase enzyme and *Kluyveromyces marixianus* maintained at 50°C at pH 3 showed the highest ethanol yield of 17.72 % and lowest yield (10.40 %) in the fermentation samples containing *Zymomonas mobilis* at room temperature (25°C) (Table 8 & 9).

Table 8
Ethanol yield estimation - Citrus maxima pulp

Pectinase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25°C)	0.22%	19.04%	0.22%	8.93%
Kluyveromyces marixianus (25°C)		14.21%		10.10%
Kluyveromyces marixianus (50°C)		19.33%		18.75%
Zymomonas mobilis (25°C)		13.91%		12.45%
Control		17.28%		15.52%

Table 9
Ethanol yield estimation - Citrus maxima pulp

α-Amylase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25°C)	0.22%	12.01%	0.22%	11.42%
Kluyveromyces marixianus (25°C)		11.42%		11.57%
Kluyveromyces marixianus (50°C)		17.72%		15.08%
Zymomonas mobilis (25°C)		12.01%		10.40%
Control		12.59%		14.35%

Citrus maxima peel - Reducing sugar analysis

Citrus maxima peel was fermented using two different enzymes (pectinase and α-amylase) and three different micro-organisms (*Saccharomyces cerevisiae, Kluyveromyces marixianus* and *Zymomonas mobilis)* were used individually. Pre-treatment with hot water and with both enzymes individually showed an increase in reducing sugar levels. The reducing sugar showed gradual decrease followed by fermentation indicated that the sugar was converted to ethanol efficiently when treated with all the microorganism and at respective temperatures. This was not varying with respect to any organism, pH or enzyme used (Table 10 & 11).

Table 10
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After pre treatment (mg)	After pectinase treatment (mg)	After Fermentation (mg)	Initial (mg)	After pre treatment (mg)	After pectinase treatment (mg)	After Fermentation (mg)
Saccharomyces cerevisiae (25°C)	0.09	0.22	1.8	0.18	0.07	0.16	2.3	0.19
Kluyveromyces marixianus (25°C)	0.08	0.09	4.2	0.20	0.06	0.28	2.4	0.21
Kluyveromyces marixianus (50°C)	0.09	0.18	1.9	0.13	0.07	0.15	2.0	0.18
Zymomonas mobilis (25°C)	0.07	0.21	2.0	0.11	0.08	0.16	1.3	0.22
Control	0.08	0.17	1.4	0.16	0.08	0.22	2.0	0.20

Table 11
Reducing Sugar Analysis – Dinitro salicylic acid (DNS) estimation

Treatment	pH 3				pH 4			
	Initial (mg)	After pre treatment	After – α-Amylase treatment (mg)	After Fermentation (mg)	Initial (mg)	After pre treatment	After – α-Amylase treatment (mg)	After Fermentation (mg)
Saccharomyces cerevisiae (25°C)	0.05	0.37	1.6	0.09	0.07	0.18	2.0	0.22
Kluyveromyces marixianus (25°C)	0.07	0.11	2.2	0.10	0.06	0.12	4.0	0.18
Kluyveromyces marixianus (50°C)	0.08	0.46	8.4	0.05	0.06	0.16	5.9	0.08
Zymomonas mobilis (25°C)	0.06	0.14	2.9	0.07	0.06	0.07	2.1	0.14
Control	0.06	0.33	3.0	0.05	0.07	0.23	1.1	0.10

Ethanol yield

Citrus maxima peel showed an initial bioethanol yield of 0.15 % at pH 3 and pH 4. There was a significant increase in ethanol percentage in the reactions containing pectinase and α-amylase enzymes at both pH 3 and pH 4. The highest yield of ethanol was obtained at pH 3. The reactions containing pectinase enzyme and *Kluyveromyces marixianus* maintained at 50°C at pH 3 showed the highest yield of ethanol (24.61 %) followed by the second highest yield of ethanol of 23.73 % of fermentation samples containing α-amylase enzyme and *Zymomonas mobilis* at room temperature (25°C) at pH 3. The combinations of α-amylase with *Kluyveromyces marixianus* maintained at room temperature (25°C) at pH 4 showed the lowest yield of bioethanol (10.69 %) (Table 12 & 13.

Table 12
Ethanol percentage Estimation: Citrus maxima peel

Pectinase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25°C)	0.15%	18.31%	0.15%	16.84%
Kluyveromyces marixianus (25°C)		16.55%		14.35%
Kluyveromyces marixianus (50°C)		24.61%		17.28%
Zymomonas mobilis (25°C)		17.43%		15.23%
Control		19.33%		15.52%

Table 13
Ethanol percentage Estimation: Citrus maxima peel

α-Amylase	pH 3		pH 4	
	Initial	Final	Initial	Final
Saccharomyces cerevisiae (25°C)	0.15%	13.47%	0.15%	13.33%
Kluyveromyces marixianus (25°C)		13.91%		10.69%
Kluyveromyces marixianus (50°C)		15.08%		12.01%
Zymomonas mobilis (25°C)		23.73%		11.86%
Control		13.62%		12.59%

DISCUSSION

In the current study an attempt was made to standardize various parameters to increase the production of bioethanol from fruit pulp and peel waste. *Citrus limetta* pulp, *Citrus maxima* pulp and peel were selected. An effective pretreatment method was standardized. Hot water pretreatment was found to be best suitable than that of dilute acid pretreatment. Pretreatment is required as the fruit waste contains more of complex carbohydrates like cellulose, hemicellulose etc. A combination of mechanical, chemical, and biological pretreatments is necessary to convert hemicellulose and pectin polymers to fermentable sugar monomers. In dilute sulfuric acid pretreatment high reaction rates can be achieved, additionally it improves cellulose hydrolysis considerably. However, when performed at moderate temperatures, direct saccharification suffers from sugar decomposition which give low yields. Hence, it is favorable to use high temperatures in dilute acid treatments of cellulosic materials. This is in accordance with our study where dilute acid (H_2SO_4) pretreatment at room temperature showed lesser reducing sugar levels as compared to hot water pretreatment with hot water at temperature. Acid pretreatment method was found to be optimal for better yield of fermentable sugars from fruit peels[17]. However, in the present study hot water pretreatment was found to be more effective than dilute acid (H_2SO_4) pretreatment. The study was also carried out to find the best suitable pH and pH3 was found to be more effective for fermentation reactions. Many studies suggested that best spirit yield was obtained with an initial pH value in the range of 4.5 – 4.7 from sugar beet juice. Soumalainen and Oure, 1971 reported that the optimum pH for inversion to be 4.0 – 5.0[18]. On the contrary, in our study, *Citrus limetta* (pulp) and *citrus maxima* (pulp and peel) showed the highest yield of bioethanol at pH 3 Two different enzymes pectinase and α amylase were used in the process of saccharification. In *Citrus limetta pulp* at pH3 there was a gradual increase in reducing sugar in the reaction with pectinase but in pH4 it was recorded that reducing sugars showed a narrow decrease following pretreatment. In the reactions containing pectinase, when the pH was at 3, the levels of reducing sugars was increased following enzyme treatment and decrease following fermentation reaction. At pH 4 there was a decrease in reducing sugars following fermentation. In the reactions containing α-amylase, the scenario was same. At both pH 3 & pH 4 there was a decrease in reducing sugar levels following fermentation. This may be due to the effective conversion of sugar into alcohol during the fermentation reaction. In *Citrus maxima* pulp and peel, treatment with hot water treatment and subsequent saccharification with both pectinase and α-amylase, showed an increase in reducing sugars and decrease in same after fermentation at both pH 3 and pH 4. This may be due to the effective conversion of sugar into alcohol during the fermentation reaction. The rate of ethanol production from *Ziziphus mauritiana* fruit pulp using was maximum at pH 6[19]. This is due to the fact that proteins function in an environment that reflects this pH (Berg, 2007). But whereas in our study the ethanol yield (19.04 %) from *Citrus maxima* pulp using *Saccharomyces cerevisiae* was obtained at pH 3. The highest ethanol yield from *Citrus maxima* pulp was obtained using *Kluyveromyces marixianus* maintained at 50^0C at pH 3. In solid state fermentation of pineapple agro residue gives a maximum yield around 2.16 % with yeast and 1.08 % by *Candida albicans* after 72 hours of fermentation[20]. Ground nut shells saccharified with *Aspergillus niger* and fermented with *Saccharomyces cerevisiae* showed maximum productio of ethanol [21.] However, in the current study a combination of *Kluyveromyces marixianus* along with pectinase enzyme gives a maximum ethanol yield of 24.61 % at pH 3 after ten days of fermentation at 50^0C.

CONCLUSION

Thus, in the current study among the fruit wastes tested it was recorded that *Citrus maxima* peel was the best source and in combination with pectinase enzyme, *Kluyveromyces marixianus* maintained at 50°C for maximum production of ethanol.

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