Research Journal of
Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (10): 763-769, 2007
ISSN 1816-4935

Production of Ethanol from Mango (*Mangifera indica* L.) Fruit Juice Fermentation

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Abstract: Scope for producing ethanol from the surplus and non-attractive mango (*Mangifera indica*) fruits was investigated. Six varieties of mango that are abundantly occurring in the region were selected for the study and the physico-chemical properties of mango was evaluated. The mango juice from selected varieties contained 18-20% Total Soluble Solids (TSS) and 5-18.5% of reducing sugars. Finally 8.5-10% (w/v) of ethanol was obtained from the fermentations which were conducted without adding any nutrients. The fermentation was completed within 72 h in all variety juices. Fermentation process optimized and pH 5.0, 30°C temperature, 3% (v/v) inoculum density and 3 days incubation was found be good for maximal ethanol production from mango juice.

Key words: Mango juice, ethanol production, waste utilization, optimization, characterization

INTRODUCTION

Ethanol is a clean burning, renewable resource that can be produced from fermentation of glucose rich substrates (Yu and Zhang, 2004). In many parts of the world, demand for ethanol as an alternative fuel source has steadily increased (Sheoran *et al*., 1998) due to efforts in decreasing the overall amount of greenhouse gases emitted into the atmosphere (Martin *et al*., 2002), dwindling fossil fuel resources, (Yu and Zhang, 2004) and increased gasoline prices. Since 1970s, it has become clear that availability of domestic natural gas and petroleum cannot meet the growing demand for these energy sources. Therefore, there has been serious concern for developing renewable energy sources in an effort to ease the severity of the expected shortage. One possibility is the conversion of waste or grown organic matter into liquid and gaseous fuels (Graham *et al*., 1976). Currently, the United States produces approximately three billion gallons of ethanol from corn annually (Potera, 2004).

Mango (*Mangifera indica*) is the most important fruit crop of the country, India occupying about 60% of the total area under fruits. The total area under mango is estimated at 2 million acres. It has been cultivated in India for at least 4,000 years. The mango fruit is a large drupe exceedingly variable in form and size, soft or juicy; sub acid or sweet with rich in aromatics and the seed is solitary, ovoid-oblique encased in hard compressed fibrous endocarp (stone). It has rich luscious, aromatic flavor and a delicious taste in which sweetness and acidity delightfully blended. It contains good amount of sugar (16-18% w/v) and many numbers of organic acids and also a good antioxidant, carotene (as Vitamin A, 4,800 IU). Some of the varieties are very rich in sugar, it s recorded as 20.5% in Nilambari. Sucrose, glucose and fructose are the principle carbohydrates in ripe mango with small amount of cellulose, hemicellulose and pectin. The green tender fruits are rich in starch, during ripening the starch which is

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present is hydrolyzed into reducing sugars(Anonymous, 1962). The unripe fruit contains citric acid, malic acid, oxalic acid and succinic and other organic acids. In ripun fruits the main acid source is malic acid (Giri *et al*., 1953).

In India, an effort to better appreciation of food processing industries, the nodal ministry, Ministry of food processing (MEPI) suggests alcoholic drink from non-molasses. Waste mango is defined as fruit that does not meet domestic and external organoleptic standards for acceptability on the international market. Waste mangoes include undersized, those with spotty or marked peel, or those that have suffered mechanical damage. Mangoes grown for export are typically harvested and transported to a packing shed for grading. The grading rejects then require disposal. Because they have already been collected and brought to a centralized location, the waste mangoes are low-cost, concentrated biomass feedstock. Such mangoes can be used as raw material in the generation of byproducts with increased added value, thus allowing farmers to diversify their harvests and receive higher income. In this context attempts for utilization of the surplus of mango fruits which are unattractive and of small size several applications have been studied, in which one of the possibility is ethanol production.

In the present investigation, the suitability of waste mango fruit juice for ethanol production was studied. We concentrated mainly on maximize the high quality juice extracted by improving the extracting techniques and optimize the fermentation process to improve the volumetric productivity of ethanol.

MATERIALS AND METHODS

Mango Fruits

Six varieties of mangoes were selected from the available ones grown in the region of south India, Table 1. The mangoes are purchased from local market (in the year of 2006) of Tirupati Andhra Pradesh, India.

Processing

Mangoes were washed with 1% HCl water and peeled off manually. The pulp was divided into two portions. The first portion was left as control and the subsequent samples of the second portion were treated with various concentrations of Trizyme P50 pectinolytic enzyme (procured from Triton Chemicals Mysore), in order to determine the most suitable and effective amount of enzyme and treatment time. In all the above treatments 200 mL of pulp in 500 mL conical flask was placed on a

Table 1a: Chemical characteristics of juice produced from different mango varieties

Variety	Sugars (%) (w/v)	Titrable acidity (%) (TA)	pH	TSS (%)
A. Banaesha	18.0	0.320	4.5	20.1
Alphanso	16.3	0.350	4.1	16.0
Banglora	16.0	0.310	4.2	16.5
Banginapalli	18.5	0.326	4.0	20.5
Neelum	15.0	0.471	3.8	15.5
Raspuri	15.5	0.430	3.9	14.2

* Values presented is mean of triplicate experiments

Table 1b: Physical characteristics of wine produced from different mango varieties

Variety	Alcohol (%) (w/v)	Sugars (%) (w/v)
A. Baneshan	8.0	2.0
Alphonso	7.5	2.1
Banglora	7.0	2.0
Banginapalli	8.5	2.0
Neelum	6.5	2.5
Raspuri	7.0	2.4

*Values presented is mean of triplicate experiments rotary shaker for intimate mixing. Juice extraction was made by pressing the treated pulp in cheese cloth and the yields of juice were compared. The juice obtained in this manner was then subjected for the analysis of total and reducing sugars, total acidity, pH and soluble solids content. None of the varieties was ameliorated with sucrose.

Microorganism

The ethanol producing *Saccharomyces cerevisiae* 101 (wine strain) obtained from CFTRI Mysore was used in the experiments. The culture was maintained on MPYD (Malt extract 3 g L^{-1}, Peptone 5 g L^{-1}, Yeast extract 3 g L^{-1} and Dextrose 20 g L^{-1} and agar 15 g L^{-1}) slants at 4°C. The inoculum was prepared by inoculating the slant culture into 25 mL of the sterile MPYD liquid medium taken in 100 mL flask and growing it on a rotary shaker (100 rpm) for 48 h. This inoculum (3×10^6 cells mL^{-1}) was transferred to 250 mL conical flask having 100 mL sterile mango must (juice).

Fermentation

Batch fermentation of the inoculated must was carried out in a number of flasks in order to determine the optimum pH and temperature by incubating at different pH (3.5, 4, 5 and 6) and at different temperatures (20, 25 and 35°C), respectively over a period of 3 days. The samples were collected by separation of the cells by centrifugation at 5,000 g for 10 min. The samples were kept at 20°C until analysis.

Analytical Methods

Sugar concentration was estimated by Shaffer and Somogyi (1995) method. Sucrose was estimated by Dubios (1956) method Total dissolved solids were measured by estimating specific gravity of water soluble portion of the mash obtained by the centrifugation 10,500 x g for 15 min. The specific gravity was determined at 20°C with Densitometer. With the aid of approximate tables the results were converted to grams of dissolved solids per 100 mL and expressed as grams of sucrose.

Types of Sugars

The sugar types that are present in mango pulp were determined by paper chromatography according to Trevelyan *et al*. (1950). Sugar spots were detected with alkaline silver nitrate reagent. Mean of triplicate experiments were presented.

Ethanol and Other Volatile Compounds

Ethanol was determined with the help of Gas Chromatography (Antony, 1984). Total acidity was determined by neutralization with 0.1 N NaOH expressed in tartaric acid and volatile acidity within the distillate samples expressed in acetic acid Mg 100 mL^{-1}.

RESULTS AND DISCUSSION

Compared to the non- enzymatic method, the enzymatic extraction of pulp gave higher quantity of juice. The effects of concentration of enzyme and incubation period were shown in Fig. 1a and b. A maximum yield of juice from the mango pulp was obtained in case of 0.6% v/v of pectinolytic enzyme with10 hour treatment period. These results were in accordance with previous investigations relate to wine and apple cider production. It is well known that pectinase degrade the pectin and liberates more juice from pulpy fruits like mango and banana (Joshi and Bhutani, 1991). In addition to this pectinase treatment decrease the viscosity of the juice and increase the fermentation rate during ethanol fermentation (Joshi and Bhutani, 1991). The total sugar present in the different mango varieties varied according to the variety and ranges from 16-18% (w/v) (Table 1a). Sugar content is different

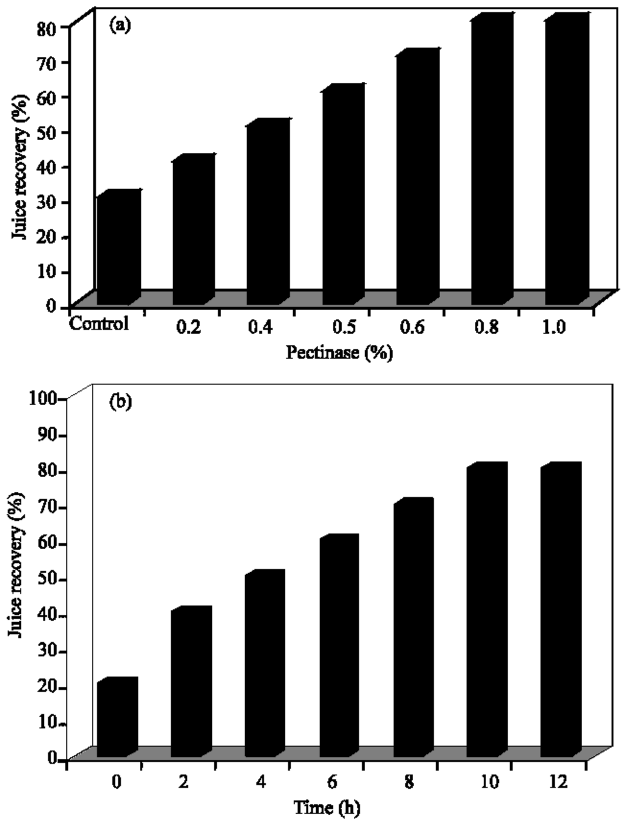

Fig. 1(a): Optimization of pectinase enzyme concentration for maximum recovery of juice from mango pulp and (b) Optimization of pectinase enzyme incubation time for maximum recovery of juice from mango pulp

from different variety of fruits and mainly depends up on the soil type and irrigation levels (Kulkarni *et al*., 1980). With the aid of paper chromatography the sugar types were identified, mango contained only 3 types of sugars, namely glucose fructose and sucrose, the major part of sugar being sucrose (Fig. 2). The main sugar from all the fruits is sucrose and followed by glucose and fructose. In mango fruits fructose is in high content s compared to glucose, although the starch is the main carbohydrate at unripe stage. Starch and sucrose are degrading to glucose and fructose during ripening of the fruit. Due to the good amount of reducing sugars available from the mango fruits it is possible to produce good amount of ethanol by economically cheap through mango juice fermentation. In general the sugar concentration from 15 to 20% (w/v) is preferable in industrial ethanol productions. It has been proved that the high substrate content during ethanol production slowdown the fermentation rate due to the increased osmotic stress (Reddy and Reddy, 2005).

The pH of the six selected mango juice varieties is between 3.8-4.5. The low pH 3.8 was found in Neelam (Table 1a). The physico-chemical characteristics of mango juice after the fermentation were as shown in Table 1b. The percentage of alcohol was between 7-8.5% (w/v). The ethanol produced from mango juice fermentation is near to the industrial level (Hammond *et al*., 1996). The ethanol percentage mainly depending on sugar which is present in the juice we used. All the sugar available was used by the organism and very little residual sugar was remained in the media while completed the fermentation. These results suggest that there are no inhibitory components in the mango juice which

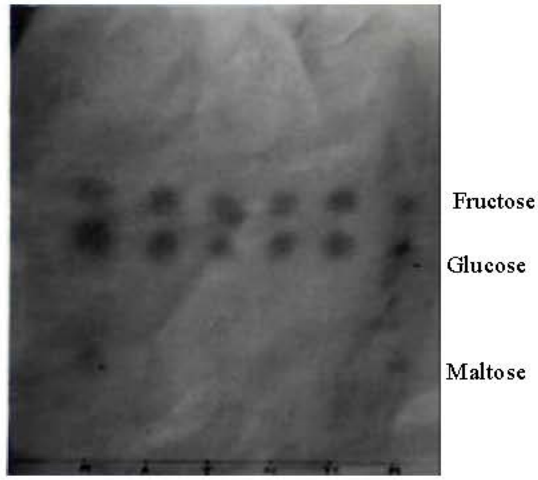

Fig. 2: Chromatogram showing types of sugars present in mango fruit

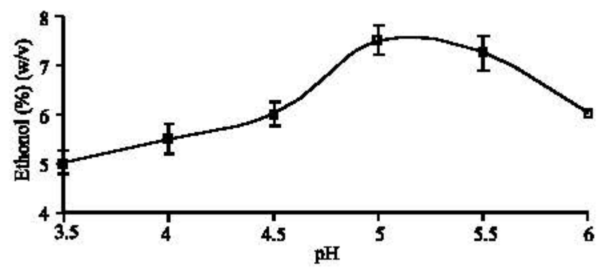

Fig. 3a: Effect of pH on ethanol production from mango pulp

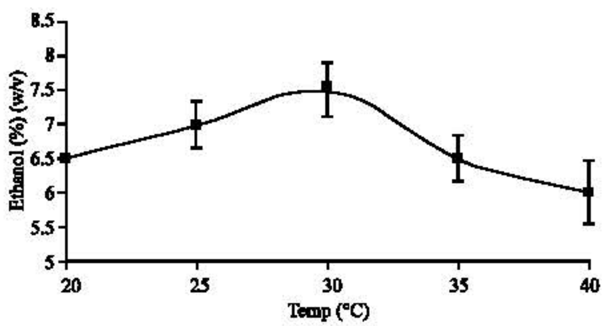

Fig. 3b: Effect of temperature on ethanol production from mango pulp

is one of the advantages for ethanol production. The pH of the produced wine had slightly increased when compared with the mango must. This increment in pH after fermentation may be because of yeast metabolites which are released into the medium during the fermentation. The culture conditions like pH, temperature and inoculum size were optimized. The effects of pH and temperature on ethanol production from mango juice were showed in Fig. 3a and 3 b. From the Fig. 3a and b it is clear that at pH 5.0 and temperature 30°C ethanol production was high and also optimum conditions for mango juice fermentation. The optimum pH of ethanol fermentation is very close the mango juice pH. In addition to this, optimum pH is also near to the room temperature so need to adjust the pH and special care for temperature maintenance. None of the varieties were supplemented with nitrogenous sources like yeast extract, peptone and malt extract. Mango juice contains around 0.6% proteins (data was not presented) that only utilized by the used yeast, *S. cerevisiae* 101. This property is very advantageous and intern it could reduce the ethanol production cost from mango juice.

Cost Economics

Around 2 kg of mangoes are requires for 1 L juice. The waste mangoes are freely available we expend only transport charges to gather at one place and the processing cost. Hence we calculated approximately 0.5 $ (US) is enough per liter ethanol. However scale-up studies need to be carried out for knowing concrete cost value of ethanol from mango juice.

CONCLUSIONS

Pectinolytic enzyme treatment is required to obtain high yield of juice from mango pulp. Fermentability also increased significantly with pectinase treatment. Banginapally variety is giving high ethanol compared to all other varieties. Fermentation conditions are also optimized and the selected yeast stain, *Sachharomyces cerevisiae* 101 is very much suitable for ethanol production from mango fruit juice. Using waste mangoes for ethanol production appears very promising. Ethanol yield (on a dry weight basis) from mangoes is higher than from most other agricultural wastes. The mangoes which, currently treated as a waste and as such, represent a very low cost feedstock. They ferment well and have a high carbohydrate concentration. Waste mangoes are already collected at packing areas and therefore transportation/harvesting costs will be minimal. No need of water for dilution and fermentation results in some what high concentration beer that would minimize distillation energy costs. Finally we conclude that mangoes are very important choicest fruits in India and an attempt to use this fruit in ethanol production would contribute considerably to the economy of the Indian as well as international mango producers.

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