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Short Communication

Fermentation and characterization of wine from fruits of *Phoenix dactylifera*, using *Saccharomyces cerevisae* NCIM 3495

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Abstract

Dates i.e. fruits of *Phoenix dactylifera*, have tremendous nutraceutical properties. These are rich in minerals, assimilable sugars, water soluble vitamins and fibers. The fruits are consumed to regain vitality which may be lost due to overexertion like in case of field laborers mostly in tropical countries. It can be used to treat spleen disorders since it is rich in iron and antioxidants. In this study wine from dates was prepared using *Saccharomyces cerevisae* NCIM 3495. The incubation was carried for 21 days at 12°C and further 8 days at 28°C. The wine has Radical Scavenging Activity of 74.53% and assimilable iron as 40%. It was free from fuselols like amyl alcohol, thus avoiding bottle ageing. The sensory values show good acceptability to discriminating consumers. If consumed moderately (like all wines) it serves as a prophylactic measure for many oncogenic diseases as well as for circulatory disorders like arteriosclerosis. Since the wine has a phenolic and flavonoid content of 16 mg/ml, which can have good immunostimulatory effect.

Introduction

Dates are the sweet fruits of the tree *Phoenix dactylifera* or the date palm. These fruits are being relished all over the world and virtually at all age group of people, primarily as a source of energy. Moreover, these are consumed by majority in the warmer regions of the planet and hence the date palm is grown in such regions (Morton, 1987). The nutritional value of the fruit is well known as it is rich in water soluble vitamins of B group, which is often lost due to exhaustion involving manual labor. It is also rich in many minerals specially potassium, calcium and iron, along with many antioxidants, and flavonoids (Habib and Ibrahim, 2011). Its high content of dietary fibers, often makes it a good food supplement for hyperglycemic patients (Vayalil, 2012). Besides these, the dates are cholesterol free, fat free and sodium free.

Usually the dates are consumed directly, or in dehydrated forms like pitted dates. These are also used as animal feed; especially camels in the middle eastern countries are often given fodder supplemented with dried date pieces (Walid and Richard, 2003). Very little knowledge is available on alcoholic beverages being produced from this fruit. The high sugar content of the fruit has been commercially exploited to produce vinegar directly (Das and Sarin, 1936). It has been reported that dates and peppers are added to native beers in Nigeria to reduce its intoxication.

In this study, an attempt has been made to, produce fruit wine from locally available dates. Fruit wines are known to have medicinal values, like radical scavenging activity and anti microbial activity, besides being a good source of certain nutritional elements like vitamins and essential minerals. The free radicals circulating in blood can be considered as a predisposing factor for many oncogenic diseases, as these can bring about damage to DNA. These are also responsible for diseases like arteriosclerosis by bringing about oxidation of the cholesterol and depositing it in the inner walls of arteries. One of the authors has produced 3 such fruit wines from dried apricots, dried grapes and dried figs (Bapat, Jadhav and Ghosh, 2010; Kadam, Upadhye and Ghosh, 2011). It was observed that the radical scavenging activity (RSA) was between 74 to 76% in these wines. It was also noted that many people preferred to drink fruit (other than grape) wine over the regular wines prepared from white and red grapes. In countries like India there are consumers who are taking to drinking wines, due to greater awareness of the fact that wines are in fact good for health when consumed moderately. It is said that a goblet or 2 of wine a day is essential for good health (Mukamal *et al*., 2008). At the beginning they start with drinking the port wines since these are very sweet and invariably most of the fruit wines are sweet too. Secondly, varieties of wines made from grapes (at affordable cost) are also limited in number, in many developing countries. The good wines which

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has been properly aged and ripened are still meant for the affluent class of citizens as these are usually imported from other countries. On the other hand, if one keeps in view of the medicinal and nutritional values of wines, then fruit wines have a good market. The production of fruit wines from fruits like apples, peaches etc., have been reported earlier, from other countries too (Robinson, 2006). However, in tropical countries and more so in rain fed regions, there are a large number of fruits which are either cultivated from the agronomic point of view, or is found in the wild (which are normally berries). The best way to get the nutritional values is by eating these fresh fruits. The paradox is that these fruits are seasonal, mostly abundant immediately after the rains or at a particular season. The only way is to prepare beverage like wine which are moderately alcoholic in nature and would make the nutritional elements available ubiquitously throughout the year. In this study an attempt has been made to prepare wine from dates (not dried) and check its nutraceutical characteristics along with sensory evaluation.

Material and Methods

Selection of fruit

The well ripened and handpicked date fruits were bought from the local market. These were deseeded and cut into small pieces and 20 g of these pieces were added in 100 ml distilled water and were homogenized in a blender. Ammonium nitrate was added to this at 0.5% level. The must thus obtained was filtered prior to pasteurization. The pasteurization process was carried out at 110°C for 15 min and then cooled rapidly to 5°C.

Microorganism

Saccharomyces cerevisiae NCIM 3495 were grown and maintained on GPYE medium containing Glucose 0.5%, Peptone 0.5% and Yeast Extract 0.3% with pH 5.5. Inoculum was prepared in the same must (as mentioned above) that was used for final fermentation. The incubation was done anaerobically at 28°C for 48 hrs. The cell density was approximately, 4.8 x 10^8 cells/ml. and alcohol content was approximately 2%. This was used at 10% level in the final fermentation medium. Two sets of experiment were set up. One was incubated at 12°C for 21 days and then at 28°C for 8 days. The other flask was incubated at 28°C only till the alcohol concentration remained constant (which was 6 days).

The growth in the latter flask was monitored at regular time interval as change in absorbance at 540 nm which was converted into number of cells per ml using the MacFarland's method (Murray *et al.*, 2007).The results were used for calculation of specific growth rate (μ) by the equation:

$$\ln (x_t/x_o) = \mu t$$

where x_t = growth at a specific time
x_0 = initial population
t = time in minutes

Simultaneously alcohol content was also monitored. Alcohol was estimated, after distillation at 78°C, by potassium dichromate method of Knox and Pask (1950). At the end of the incubation as mentioned above i.e. for 28 days (21days at 12°C followed by 8 days at 28°C), the fermented wine was centrifuged at 5000 x g for 20 minutes, to remove all cells and other debris. The clear supernatant was the used for further characterization.

Acidity of the wine was checked using 10 ml cell free wine to which 10 ml distilled water was added along with a few drops of phenolphthalein indicator. It was titrated against 0.1N NaOH. The quantity of tartaric acid and acetic acid was calculated as follows:

% of Tartaric acid = ml of alkali x Normality x (7.5/ wt. of sample)

% of Acetic acid = ml of alkali x Normality x (6.0/ wt. of sample)

Reducing Sugar content, (both before after fermentation) was determined by dinitrosalicylic acid method (Miller, 1959).

Antioxidant activity was assayed as percentage radical scavenging activity (RSA), using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent (Ko *et al.*, 1998).
It was calculated as:

% RSA = (Acontrol – Asample) x 100 / Acontrol

Where, A = absorbance at 517 nm.

Total soluble solids (TSS) content

In this method a known volume of the must and fermented wine were centrifuged separately at 5000 x g for 20 mins, to remove all insoluble matter. The clear supernatants were then evaporated at 60°C (to prevent charring and Malliard reaction). It was then cooled to ambient temperature and weighed. The procedure was repeated till constant weight was obtained.

Determination of fat content

Homogenised date (10 g) was progressively added to small amounts of a chloroform / methanol 2:1 (v/v) mixture (up to 200 ml), with vigorous shaking, and then the extraction was carried on for a further 2 h, with constant stirring. This was filtered and the residue was re-washed with fresh solvent and pressed. Fifty milliliters of 0.88% potassium chloride was added to this filtrate with constant stirring. The aqueous layer (upper) was removed by aspiration and the procedure repeated a couple of times. This was dried by passing through anhydrous sodium sulfate, and then the solvent was removed by using a rotary evaporator. The residue was weighed to find the fat content.

Estimation of ash content

The ash content of date sample was measured by incinerating it in Muffle-furnace at 560°C. The total ash content was calculated by using this formula.

Total Ash content (% of ash) = (Weight of ash/ Weight of sample) x 100

Determiation of total Phenolic / flavonoid content

The total phenolic content of wine was determined by spectrophotometrically according to Folin-Ciocalteu method as described by (Malick and Singh, 1980; Chang *et al*., 2002).

Determination of total carbohydrate content

Chemical analysis for the determination of total carbohydrate was checked by the phenol–sulphuric acid method (Dubios *et al*., 1956).

Measurement of Iron (as Ferric ions)

The total concentration of iron from wine sample was measured spectrophotometrically (Goswami and Kalita, 1988).

Biochemical characterization

Samples for these studies were prepared by drying 1 ml of wine. The dried sample was extracted in absolute ethanol. This extract was used for HPLC analysis using C-18 column.

Total protein analysis

The total Protein Analysis was carried out by Biuret method (Layne, 1957).

Results and Discussion

It can be seen from Figure 1, that the yeast shows typical diauxic growth, utilizing the monosachharides first then the other sugars. Though the fruit is rich in polyphenols, the presence of fermentable sugars in high percentage (to the tune of 13%) removes all stress from the growth of the yeast. The final alcohol concentration is 5.6%, which is a sign of good fermentative activity. Figure 2 shows the specific growth rate during the log phase of the growth which is 0.46023 and the R^2 value of 0.995 clearly indicating that the yeast was not growing under stress during the fermentation.

Table 1 summarizes the results of the study, wherein one can see that the nutraceutical properties of the wine like the RSA value of 74.53, along with iron content of 40 mg/ml and phenolic content of 16 mg/ml in the final product, is an indication that the wine can serve as a prophylactic agent for many diseases including certain oncogenesis and cardiovascular diseases. The observation concurs

Table 1. Summary of results of the wine fermentation

Specific growth rate (at 25°C)	0.46023
Alcohol produced (%)	5.5
Specific product formation rate	5.5×10^{-5}
Final pH	5.8
Temperature:	
21°C	21 days
25°C	8 days
Yield of biomass/unit substrate utilized	1×10^8 cells/mg of sugar utilized
Sugar concentration:	
Initial sugar concentration in the must	13mg/ml (as reducing sugar)
Residual sugar concentration in the wine	9.4mg/ml
Final Biomass concentration	1000×10^8 cells/100 ml
Protein concentration:	
Initial protein concentration in dates	400mg/ml
Final protein concentration in wine	60mg/ml
Phenolics / flavonoid concentration of the wine:	
Initial phenolics / flavonoid concentration	53.5mg/ml
Final phenolics / flavonoid concentration	16.0mg/ml
Iron concentration:	
Initial iron concentration in dates	78mg/ml
Final iron concentration in wine	40mg/ml
Antioxidant :	74.53
%RSA Value	
Acidity:	
%Acetate	0.05
%Tartarate	0.15
Ash content	68.54% (Ash content of dates used = 83.07%)
Fat content of whole dates (%)	0.5
Fat content of wine (%)	0.04

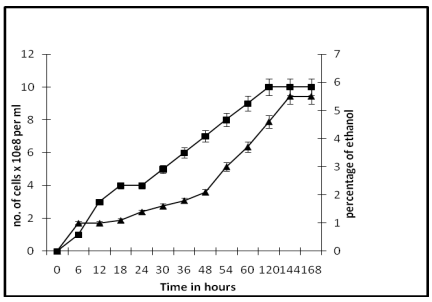

Figure 1.Growth pattern of *Saccharomyces cerevisea* NCIM 3495 in medium of date extract and ammonium nitrate incubated at 25°C under anaerobic condition. (■) growth of the organism and (▲) indicate alcohol production

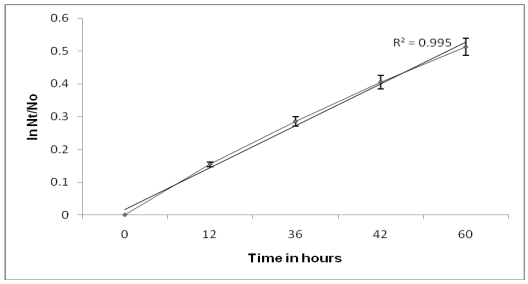

Figure 2. Growth Pattern of the organism, as seen in the second phase of the diauxic growth, (between 36 and 120 hrs) for calculation of specific growth rate ($\mu = 0.46023$). The line, not necessarily passing through any of the coordinates, is the regressed line

with the finding of other authors regarding wine prepared from apricot and raisins (Bapat, Jadhav and Ghosh, 2010) and from dried Ficus carica (Kadam, Upadhye and Ghosh, 2011). The mineral content also indicates its importance in maintaining good health of the consumer. Though there is decrease of about 30% in phenolics and flavonoids, still the wine has sufficient antimicrobial activity and that is a good indication of its self preservation nature. The wine which was prepared at 12°C for 21 days did not show presence of fuselols like amyl alcohol, which explains the redundancy of bottle ageing, which was unlike the wine prepared by incubating at 28°C for 6 days wherein some amyl alcohol was detected (results not shown). The sensory analysis on a scale of 10 also finds that the wine has an acceptable taste, aroma, color and sweetness (results not shown).

Conclusions

Date is a fruit which is easily available throught the year in most of tropical and semitropical countries. The whole fruit is not only provides the required carbohydrates (in the form of sugars) as a good source of energy, but aso many minerals and water soluble vitamins. These are mostly needed by those who do hard manual labor like field laborers in tropical countries. These people, due to excessive sweating, lose a lot of minerals and water soluble vitamins. Since they can not afford to take proper replenishment therapy due to poor economic conditions, dates serve as the best natural remedy to regain the energy and vitality. However, the quality of dates vary in those available in the market and so do the nutritional qualities. Therefore, to get uniform results ubiquitously, the best way would be to consume aloholic beverage like wine prepared from dates. Secondly, the radical scavenging activity of dates, preserved in the wine, also help to reduce the risks of suffering from oncogenic disorders due to lowered immune system. These also help to reduce the incidents of other disorders like oxidation of cholesterol which can deposit in the interior of arteries and develop diseases like arteriosclerosis leading to cardio-respiratory disorders etc. The sweet taste and other flavors of the wine makes it very attractive and acceptable to most of the consumers who are not used to consuming wine.

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