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Short Communication
Fermentation and characterization of wine from dried *Ficus carica* (L) using *Saccharomyces cerevisiae* NCIM 3282

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Abstract: Dried figs or *Ficus carica* (L) are nutritionally rich fruit. Figs are one of the highest sources of calcium and fiber along with copper, manganese, magnesium, potassium, calcium and vitamin K besides being good source of flavonoid and polyphenols including gallic acid chlorogenic acid, syringe acid and rutin. It is useful in treatment of liver and spleen disorders, to cure piles and in treatment of gout. It contains antioxidants which quenches free radical damage to cells and tissues. The study involves development of a microbial biotechnological product like wine from dried fig using *Saccharomyces cerevisiae* NCIM 3282. It was observed that after 28 days of incubation there was 4% alcohol concentration. The wine was free from fuselols like amyl alcohol, as found in HPLC analysis. It indicates that there is no need for bottle aging of the wine which is essential for grape wines. The RSA value of the wine was observed to be 76.45% indicating that the antioxidant properties are significant. It also was rich in important nutrients specially minerals essential for good health. All these go to advocate that moderate consumption of this wine can lead to better living.

Keywords: Figs, wine, antimicrobials, polyphenols, ficin

Introduction

Ficus carica is known as common fig. These are one of the earliest fruits cultivated. The fruits were dried and stored for later consumption. Legend has it that the Greek goddess Demeter first revealed to mortals the fruit of autumn, which they called the fig. Early Olympic athletes were given figs as training food and figs were given as laurels to the winners of the first Olympic as a "medal". Although considered a fruit, the fig is actually a flower inverted into it. The seeds are the real fruit in figs. They are the only fruit to ripen fully and semidry on tree. Native to areas from Asiatic Turkey to northern India, figs spread to all countries around the Mediterranean. Today the united states, turkey, Greece and Spain are the primary producers of dried figs. There are four principal varieties of cultivated figs: the amber-colored Calimyrna; the dark, purplish Mission; the small but high-in-sugar Adriatic, and the uniquely white to translucent amber Kadota. The Calimyrna and Mission varieties represent almost two-thirds of the commercial production (Vinson, 1999). Since these are now found all over the world, these are know by many common names in different parts of the world such as Anjir (parts of India), Fig (English), Higo (Spanish), Figue (French), Feige (German).

It is one of the plant sources of calcium and dietary fiber. Dried figs are rich in minerals like copper, manganese, magnesium, potassium, calcium, vitamin K, and many antioxidants relative to human needs. They are good source of flavonoids and polyphenols including gallic acid, chlorogenic acid, syringic acid, (+)-catechin, (-)-epicatechin and rutin. A 40-gram portion of dried figs (two medium size figs) produces a significant increase in plasma antioxidant capacity (Vinson, 1999). Beside these, the roots of the plant has been used to treat many dermatophytic infections like ringworms, fungal leucoderma etc. The proteolytic fraction of the leaves, which contain a compound called as ficin, is used as antihelmenthic agent (de Amorin *et al*., 1999; Shai *et al*., 2001; Canal *et al*., 2002; Asadi *et al*., 2006 and Vaya and Mahmood, 2006). The fruit has been reported to be rich in ficin which is found in the leaves too, has been proved to very effective in prevention of diseases like cancer and regulation of plasma thromboplastin and prothrombin in blood (Richter *et al*., 2002).

This study was done to see if these properties can be conserved in the form of wine which will be more acceptable as a drink rather than trying to get to the fruit. Again in many parts of the globe, this fruit may not be readily available or affordable, but then whether the wine prepared from the fruit could help to improve the health of the population. This was seen possible in a similar study on apricot and raisin wine (Bapat, Jadhav and Ghosh, 2010).

Materials and Methods

Microorganism and growth

The *Saccharomyces cerevisiae* NCIM 3282 culture was grown in medium containing Glucose 12%, Peptone 3%, NaCl 9% and incubated at 37ºC

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for 48 hrs. The organism was maintained on a media containing Glucose 0.5%, Peptone 0.5%, Yeast Extract 0.3% with pH is 6.8 to 7 (GYPE medium).

Adaptation of organism

The organism was adapted to grow at 8°C in GYP liquid medium. The culture was maintained on the same solid medium. The pattern of growth was monitored at regular time interval by taking an absorbance at 530 nm, different stages of growth are shown in Figure 2 was monitored at regular time interval by taking an absorbance at 530 nm, different stages of growth are shown in Figure 2. Specific growth rate was calculated by using the equation: $\ln x_t = \ln x_o + \mu t$

Preparation of the wine

The fruit was crushed and then coarse filtered. It was centrifuged at 4000 x g rpm for 20 min, 100 mg of food grade pectinase enzyme / 100 ml of phosphate buffer was added and kept in water bath for 3-5 hours at 30-31°C. Ammonium nitrate 0.5%, K_2HPO_4 0.01% and sucrose 12%, were added to this. This was then sterilized for 25 min. at 10 p.s.i of steam and cooled. The flasks were incubated for 21 days at 8ºC and then at 25°C temperature for 7 days. Distillation and characterization of wine was carried out after every 7 days.

Characterization of wine

Acidity of the wine was checked as:

10 ml fermented but cells separated Wine + 10 ml distilled water +Phenolphthalein indicator. Titrate it against 0.1N NaOH.

% 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

Alcohol content was checked by potassium dichromate method:

The cell free fermented medium was distilled at 78ºC. The alcohol % was estimated by $K_2Cr_2O_7$ method (Knox and Pask, 1950).

Reducing Sugar was determined by dinitrosalicylic acid method:

The reducing sugar concentration of the fermented medium was checked by dinitrosalicylic method (Miller, 1959).

Antioxidant Properties was determined as percentage radical scavenging activity (RSA %):

Antioxidant activity of the wine was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Briefly the stock reagent solution was prepared by dissolving 4.8 mg of DPPH in 20 ml methanol and stored at -20ºC until use. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance value 1.1± 0.02 at 517 nm.

The different concentrations of wine were allowed to react with 3 ml of DPPH solution. The mixture was shaken vigorously and kept at room temperature for 30 min in dark. The absorbance was measured at 517 nm. A control sample without wine was also analyzed. The results were expressed as radical scavenging activity (%RSA)

% RSA = (A control – A sample) x 100 / A control

Where, A = absorbance at 517 nm

Biochemical characterization

Samples for these studies were prepared by taking and drying 1 ml fermented wine sample, in oven. The sample was extracted in methanol. This was used for HPLC and GCMS analysis.

Results and Discussion

It can be seen from the figure 1 that the growth being very slow at a low temperature as 8°C and the presence of the polyphenols has given a very low value of specific growth rate (μ) as 0.11156. It has also been observed from the HPLC studies that by preparing the wine at low temperature there is hardly any formation of heavy alcohols like amyl alcohol. Whatever little amount that has been formed is during the 7 days of incubation at 25°C. This goes to prove the tradition that fruit wine are never to be bottle aged.

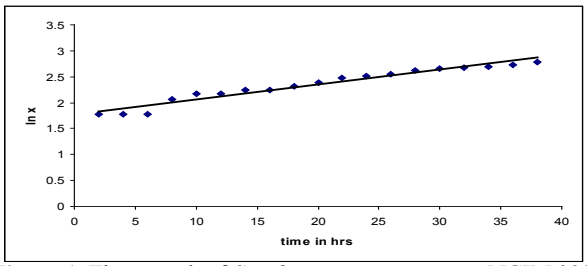

Figure 1. The growth of *Saccharomyces cerevisiae* NCIM 3282 at 8°C. The R^2= 0.9502. The slope of the line (μ) is 0.11156

The results of the GCMS analysis clearly indicated the antimicrobial agents like dibutyl phthalate, cyclopentane, 2-hexanone, furfural and Eicosanoic acid have been retained in the wine. Finally Table 1 summarizes all the results of this study. It is very evident that the cells of the organism did not only overcome the stress of the temperature but also that

Table 1. Summary of the results

μ (specific growth rate)	0.11156
p (product alcohol)	4.5%
q p (specific product formation rate)	0.00187
Final pH	5.5
Temperature 21 days 7 days	 8°C 25°C
$y_{p/x}$	0.0714
Initial sugar concentration	14%
X (final biomass concentration)	630X 10^6 cells/ 100ml
dx/dt	16.58 X 10^6
Y (biomass/substrate consumed)	13.49 X 10^6 cells/ gms
Residual sugar	3%
Acidity % of Acetate % of Tartarate	 0.09% 0.11%
Antioxidant activity % RSA	 76.45%

of the inhibitory compounds present in the fruit pulp. However, the satisfactory amount of alcohol was produced when it is compared with the residual sugar that remained in the wine. The qualitative sensory analysis shows that the "discriminating consumer" found it very acceptable as the wine had a golden brown color with a slight sweet taste and a pleasant fruity aroma. Of course it had the fig flavor.

Conclusions

Thus it can be concluded that fruit wines are excellent source of all the important pharmaceuticals and nutrients that may be needed for a healthy life. This is very evident from this study wherein it can be seen that not only the flavor and aroma of the original dried figs are retained, as consumers like the wine even more than the best quality white grape wine available in the city, because of the aroma and flavor. At the same time all the antioxidant properties along with other antimicrobial properties has been also retained in the wine. Consuming such a beverage, will never lead to addiction to alcohol, as the alcohol has been retained at a very low value of 4.5%, at the same time helping to strengthen the immune system of the consumer.

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