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Studies on the Process Development for the Fermentative Production of Wine from Grape Juice Concentrate

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Abstract

Wine is a typical alcoholic beverage produced by natural alcoholic fermentation of fruit juice of sound quality viz. grape, apple, cherry, strawberry, Raspberry, watermelon, kinnow mandarin, jackfruit etc.Wine consists of (10-12) %v/v of ethanol. The suitability of grape (Vitis rapina refers to a particular type of grape) as well as grape fruit (Citrus paradisi) juice concentrate was investigated for the production of grape wine. The significant amount of fermentable sugars (86%) e.g. glucose, galactose, sucrose, lactose etc. and acid content (.715gm/100ml of tartaric acid) present in grape juice concentrate were found suitable for its application in wine production. The fermentation was carried out by a potent yeast strain viz.Saccharomyces cerevisiae MTCC178.The grape wine is produced from reconstituted grape juice concentrate under optimized process conditions. Optimization of the inoculum volume showed that 8.5 (% v/v) inoculum contributed to the highest fermentative production rate and maximum ethanol production (%v/v).The ethanol content (%v/v) in wine was determined by spectrophotometer. Maximum ethanol concentration achieved in this study was 10.5 (% v/v) at a total soluble solids level of 20°B.Optimum temperature, time, and pH for maximum ethanol productions were found as 30°c, 7days and 5.5 respectively. The titratable acidity of final product i.e. wine of different total soluble solids viz. 10°B,15°B and 20°B were determined as .57%(v/v), .75%(v/v) and .681% respectively. The organoleptic evaluations of prepared and matured wine were carried out by 9 point Hedonic Rating Test. The sensory characterization of wine is achieved at total soluble solids level of 20°B and exhibits the superior quality in terms of its typical reddish yellow appearance, characteristic sweet aroma and a harmonious wine like taste

Key words: Grape juice concentrate, fermentation, production, wine, ethanol

Introduction

A grape is a non-climacteric fruit, specifically a berry that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes can be eaten raw or they can be used for making jam, juice, jelly, vinegar, wine, grape seed extracts, raisins, molasses and grape seed oil. Grapes are also used in some kinds of confectionery. Grapes are typically an ellipsoid shape resembling a prolate spheroid. Grapes are a type of berry that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange, and pink. "White" grapes are actually green in color, and are evolutionarily derived from the purple grape. Mutations in two regulatory genes of white grapes turn off production of anthocyanins which are responsible for the color of purple grapes. Anthocyanins and other pigment chemicals of the larger family of polyphenols in purple grapes are responsible for the varying shades of purple in red wines (Waterhouse2002, Bruillard etal 2003).

According to the Food and Agriculture Organization (FAO), 75,866 square kilometers of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit. A portion of grape production goes to producing grape juice to be reconstituted for fruits canned "with no added sugar" and "100% natural". The area dedicated to vineyards is increasing by about 2% per year. South Africa is currently ranked the ninth largest wine producer in the world, yielding 3% of the total annual production (OIV Statistics, 2005). The four largest wine producers are, in order of world ranking, Italy, France, Spain and the USA (SAWIS, 2007). The exact origin of wine is not cleared. However, used of the grape fruit for production of wine, was “domesticated” before 4,000 B.C. in Mesopotamia and Egypt. Any beverage derived from fermented fruit juice is

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considered wine. However, wine as we know it is still fermented grape juice from 6,000 years after its domestication (Rapp and Mandery. 1986).Commercially cultivated grapes can usually be classified as either table or wine grapes, based on their intended method of consumption: eaten raw (table grapes) or used to make wine (wine grapes). While almost all of them belong to the same species, Vitis vinifera, table and wine grapes have significant differences, brought about through selective breeding. Table grape cultivars tend to have large, seedless fruit (see below) with relatively thin skin. Wine grapes are smaller, usually seeded, and have relatively thick skins (a desirable characteristic in winemaking, since much of the aroma in wine comes from the skin). Wine grapes also tend to be very sweet: they are harvested at the time when their juice is approximately 24% sugar by weight. By comparison, commercially produced "100% grape juice", made from table grapes is usually around 15% sugar by weight (Wine grapes 2010). The process begins with red or white wine grapes (Vitis vinifera) that are specifically selected for the preparation of wine because of their characteristic flavors and aromas. Contrary to common belief that wine making is a relatively simple process as compared to beer fermentation. The sugar-fermenting yeasts are present in the grape skins and naturally begin fermentation whenever there is a break in the grape skin. Whereas ethanol is an alcohol made from the fermentation of the carbohydrate or sugar fraction of biomass (plant) materials (Wang et al.2005).Grapes are harvested, destemmed and crushed to form wine must – a mixture of grape juice and berry skins (reds). To initiate the process of alcoholic fermentation a selected wine yeast strain from the species Saccharomyces cerevisiae with specific characteristics, is added to the must. During the fermentation process, the yeast cells convert the grape sugars into alcohol and also produce several other chemical compounds that contribute to the final characteristics of the wine. By improving fermentation methods, the grape juice concentrate could allow fermentation of high alcohol (wines) which eliminate brandy fortification and provide an easier process to produce dessert wines. The strains of yeast (Saccharomyces cerevisiae) are mostly used for fermentations of sugar into alcohols/ Red wine, as it is commonly produced with red berried grapes. Grape juice - of every grape, including the red berried ones. The wine maker could decide about adding specific yeast cultures in order to have a better control over fermentation as well as giving the wine particular organoleptic qualities. The main and fundamental role of alcoholic fermentation (or primary fermentation) consists in transforming the sugar contained in the must into alcohol and carbon dioxide - a job done by yeasts - however this process has the purpose of developing the secondary aromas of wine as well (Ferreira et al.1995b).The duration of fermentation and maceration depends on the style of wine to be made as well as on the varieties of grapes. In light bodied wines this phase can last three or four days - a sufficient time in order to extract color and some tannins - whereas in full bodied wines this time can also have duration from one week to one month. The process of alcoholic fermentation will be completed in most of the cases, within one week. Maceration is a the phase in which tannins and gustatory substances are being extracted, is a process that must be scrupulously controlled because excessively long times could result in the extraction of an excessive quantity of polyphenols therefore obtaining very astringent wines with a bitter and mediocre taste. At the end of maceration the wine is separated from the skins and transferred in the aging containers (Park, 1991). As opposed to white wines, in red wines malolactic fermentation (The process in which malic acid is converted into lactic acid and carbon dioxide) is always done. In red wines malolactic fermentation always plays a positive role because it makes the wine smoother and less astringent. Malic acid accentuates the astringent effect of tannins therefore its transformation into a “sweeter” acid, such as lactic acid, greatly contributes to the roundness of wine (Bai et al.1994).The majority of aromatic compounds founded in grape and grape wine have simpler structures. The contents of these organic compounds are organic acids esters, hydroxybenzene and terpene etc (Schreier1979; Sften et al. 1993)).Furthermore, the odor of wine is due to four esters (ethyl acetate, isoamyl acetate, ethyl hexanoate and octanoate) along with two alcohols, (isobutyl and isoamyl alcohol) and acetaldehyde, all of which are fermentation products (Ferreira et al. 1995b; Rapp and Mandery, 1986; Perez et al. 2003).The volatile components of wine are considered as the basic fragrance when supplemented to the wine can improve the quality of wine (Avakyants et al.1981; Falque et al.1995).A major breakthrough for grapevine characterization and improvement was the sequencing of the entire genome by researchers in France and Italy in 2006 (Jaillon et al., 2007; Velasco et al.,2007). Genetic engineering has enormous application potential in the improvement of grapevine, based on the market demand for cost-effective, sustainable and environmentally friendly production of healthy, top quality grapes and wine (Vivier and Pretorius 2002).The quality of wine is known to depend upon a number of factors like cultivars and their characteristics such as adequate sugar level, acid content, color and aroma (Ethiraj and Suresh 1993).The method of wine production also affects the quality of wine(Timberlake and Bridle 1976).Few would dispute that the key element to red winemaking resides with the quality of fruit, and that there is a close link between wine color and wine quality (Somers and Evans 1974;Gishen et al. 2002). Much research has been dedicated to understanding the chemistry of grape pigments so that management tools and strategies can be developed to assist in the growing of grapes and production of wine for optimum color. The large amount of fermentable sugars, acid content and characteristic flavor makes it suitable for wine making. So far grape juice concentrate is preferable for winemaker to produce wine

continuously throughout the season due to its cost minimization, easy transportation and storage. Therefore keeping in view of all these facts the current work has been carried out to investigate the novel technology for the process development of wine production from grape juice concentrate.

Material and Methods

Fresh and fully matured grape fruits were selected and procured from the local market of Sodepur (West Bengal), India in the month of April to May. The agar slant culture of Saccharomyces cerevisiae MTCC 178 was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. All the chemicals used in this investigation were of analytical grade, except cane sugar or sucrose, procured from the local market. Sterilizable polypropylene screw capped glass bottles were used during the entire research work. The agar slant cultures of yeast specimen were activated according to the prescribed procedure by the MTCC and were suitably grown in sterile Yeast Extract Peptone Dextrose broth (YEPD). The supplied culture was maintained by transfer on Glucose Yeast Extract Agar plates and stored to refrigeration temperature i.e at 4±1°c, until further use. These were sub cultured minimum of 2-3times before use. Fresh and matured grape fruits were cleaned thoroughly with running water thereby removal of adhering dust and dirt, washed, air dried and finally packed in polyethylene bags for further use. Grape juice was extracted through a screw type juice extractor (Laboratory Model). The extracted juice was filtered and juice concentrate was prepared accordingly. The juice concentrate was analyzed for the physico-chemical parameters viz. pH(Digital pH meter),Total soluble solids(by Hand Refractometer,ERMA, Japan), titratable acidity(Amerine and Joslyn 1950;Amerine and Ough 1980;Gallander etal 1987) and reducing sugar and total sugars(Lane and Eynon) before its utilization for the preparation of wine. Grape juice concentrate was reconstituted by the addition of distilled water. Its total soluble solids (°Brix) and pH was adjusted to 10°B, 15°B and 20°B and 5.5 respectively. Diammonium hydrogen phosphate (0.1%) and SO2 in form of potassium metabisulphite (100ppm) were added to it before fermentation. The reconstituted grape juice concentrate was again filtered and pasteurized 85°c for 10 minutes at 1 atm and then immediately cooled and stored at 4-5°c until further use. A loop full of culture of Saccharomyces cerevisiae (MTCC 178) was transferred in sterilized, reconstituted grape juice concentrate. All the culture were grown at 25°c under agitation rate (150r.p.m) for 48h and 8.5 %(v/v) inoculums was used for the preparation of wine. The effect of total soluble solids(10-22°B), initial pH(4.0-6.0), temperature of fermentation(20-35°c), inoculums size(7-9%v/v) and time of fermentation(3-10)days on alcoholic fermentation of reconstituted grape juice concentrate by Saccharomyces cerevisiae MTCC 178 was investigated. The grape wine was prepared according to the method shown in Figure 1. The wine was analyzed for total soluble solids (°B), total and reducing sugars, titratable acidity, pH and ethanol concentration. Ethanol in wine was measured colorimetric ally (Caputi etal 1968). Sensory evaluation of the prepared wine samples was done by 9 point Hedonic Scale (Rangana 1986) through a group of semi trained panelist.

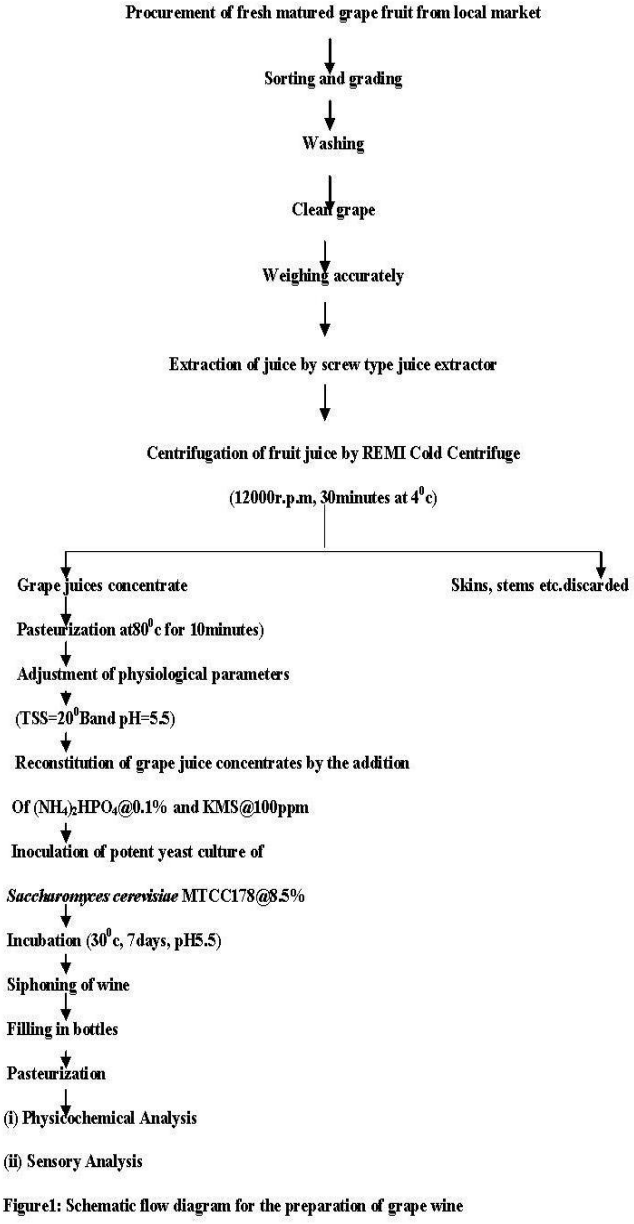

Figure1. shows the schematic flow diagram for wine production.

Physicochemical characterization of grape juice concentrate. Physicochemical characteristics of the juice were analyzed and total sugars, reducing sugars and glucose came as 90%, 82% and 45% respectively. Titratable acidity (%tartaric acidv/v) and pH was found to be .715 and pH was .715% and 5.0 respectively. The large amount of fermentable sugars (86%) and acid content (.715%) in the grape juice concentrate were found suitable for its use in preparation of wine. The reconstituted grape juice concentrate is resistant to microbial deterioration and its shelflife is significantly increased.

Effect of total soluble solid concentration (0B) on ethanol yield (%v/v) of reconstituted grape juice by Saccharomyces cerevisiae MTCC 178.

The effect of total soluble solid on ethanol yield was studied by carrying out at variable soluble solid concentrations ranges of (100B-220B). The results were shown in figure 2. It was evident from the result that an increase in ethanol production was recorded with the increase in total soluble solids up to 220B and thereafter no change was recorded for this parameter. So 200B was considered as optimum for further study.

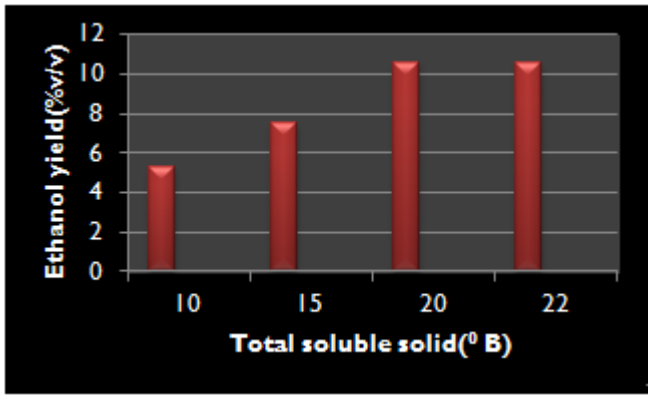

Figure 2. Effect of total soluble solid concentration (0B) on ethanol yield (%v/v)

Standardization of environmental conditions for the production of ethanol from reconstituted grape juice concentrate by Saccharomyces cerevisiae MTCC178. Effect of pH on ethanol yield (%v/v) of reconstituted grape juice by Saccharomyces cerevisiae MTCC 178.

Different pH was tested for the production of ethanol from grape juice concentrate. Experimental studies showed (figure 3) that up to pH 5.5 maximum alcohol yield was achieved and after that there was a slight drop in alcohol yield. Therefore pH of alcohol fermentation 5.5 was considered optimum for future course of work.

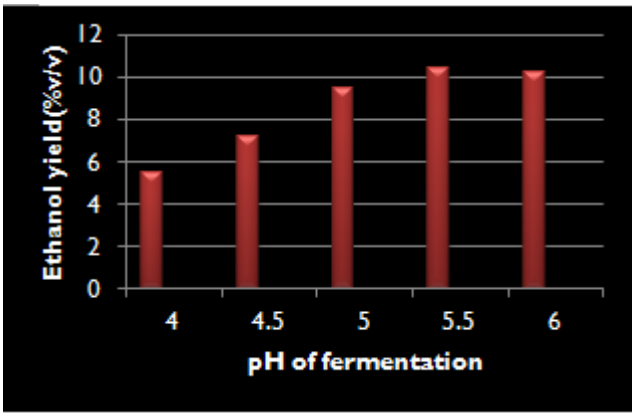

Figure 3. Effect of pH of fermentation on ethanol yield (%v/v)

Effect of time of fermentation on ethanol yield (%v/v) of reconstituted grape juice by Saccharomyces cerevisiae MTCC 178.

Ethanol production was carried out by varying time of fermentation ranges of (3-10) days. The results were shown in figure 4 Maximum production of ethanol was achieved at fermentation time 7 days. Afterwards there is a significant decrease in alcohol yield. Hence 7 days time was considered as optimum for further study.

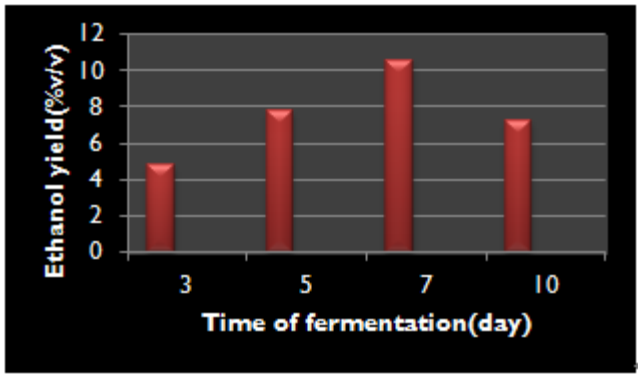

Figure 4. Effect of time of fermentation on ethanol yield (%v/v)

Effect of temperature of fermentation on ethanol yield (%v/v) of reconstituted grape juice by Saccharomyces cerevisiae MTCC 178.

Alcohol fermentation of reconstituted grape juice concentrate was carried out for 7 days at a temperature range of (20-35)0c. The results were shown in figure 5.The maximum ethanol production was observed at a temperature of 300c. Comparatively ethanol production was lesser at 200c than at other temperature tried. Therefore a temperature of fermentation 300c was selected as optimum for further studies. It was reported that when the fermentation temperature is between (10-15)0c, most of the main aromatic compounds that endow the products with

wine character can be preserved (Singh, Panesar and Marwaha 1998).

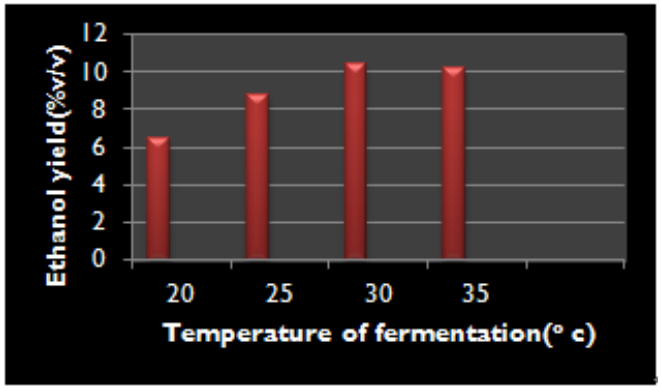

Figure 5. Effect of temperature of fermentation on ethanol yield (%v/v)

Effect of inoculum volume (%v/v) on ethanol yield (%v/v) of reconstituted grape juice by Saccharomyces cerevisiae MTCC 178.

Inoculum volumes (%v/v) were varied from (7-9%v/v), to study its influence on ethanol fermentation. The experimental results were shown in figure 6. It was evident from the observation that ethanol production was increased with the increase in inoculum concentration up to 9.5 %(v/v) for a fermentation period of 7 days at a temperature level of 300c and pH of 5.5. A slight drop in alcohol production was observed beyond inoculum level of 8.5 %(v/v). So an inoculum level of 8.5 %(v/v) was considered as optimum for furtherstudies.

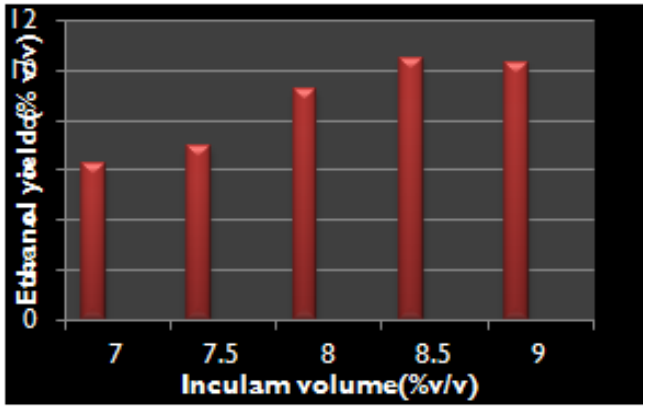

Figure 6. Effect of inoculum volume on ethanol yield (%v/v)

Physico-chemical characterization of grape wine produced under optimized fermentation conditions.

Physicochemical characteristics of wine after the completion of fermentation under optimized environmental conditions were studied. Ethanol content, residual sugar concentration and total soluble solid were found as 10.5 %(v/v); 4 %(w/v) and 6(0B). Here ethanol is the chief component on which the type of wine can be characterized. Table wine usually contains 11-14% alcohol and may have as low as 7 %(Joshi1998). The ethanol content in wine is influenced by method of wine preparation, type of yeast used and initial total soluble solids in must ((Joshi and Sharma1994). Fruit wine aroma, widely considered to be a key aspect of quality, is the result of interaction between components of the fruits themselves and those produced during processing, fermentation and ageing and the consumers sense of smell((Sanchez etal 2005). Titratable acidity and pH of grape wine was .681% and 5.5 respectively. Titratable acidity of any fruit wine is an important characteristics varying between (0.5-1.0) %(Joshi 1998). The acidity of fruit wine is dependent upon a number of factors like type of fruit, method of preparation and type of yeast used (Singh and Kaur 2009).

Sensory Evaluation

The sensory evaluation of various quality attributes of wine samples of total soluble solid concentrations viz. 100B; 150B and 200B were carried out on the basis of 9-point Hedonic scale viz. aroma,taste,color and over-all acceptability. The wine produced from 200B total soluble solid concentration was found to maximum score and superior in quality.. It was also reported that grape wine bears clean appearance and light amber color. It was having a pleasant fruity smell of natural grape fruit and harmonious wine taste.

Conclusion

The satisfactory quality of wine greatly depends upon nature of cultivation, maturity, growing conditions of grape fruit, pedoclimatic conditions and most importantly on kinetics of yeast fermentation and potential of selected yeast strain. All these factors are responsible for the characteristic difference of flavor, taste, appearance and over-all acceptability among all types of fruit fermented wine.

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