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Production of wine from red grapes & study the effect of pomace on seed germination

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Abstract

Wine is one of the alcoholic beverage that is traditionally made by fermentation process of sugar containing fruit juice by using yeast. Wine is a safe and nutritionally beneficial alcoholic beverage. Generally, the grape juice is used as a raw material for preparing wine and *Saccharomyces cerevisiae* for fermentation. In the present research, the wine is prepared at laboratory conditions using Red grapes. The produced wine is analysed for its physical properties for every 15 days' interval *viz* pH, TRS, RS, Specific Gravity, Brix, TDS and TSS. The final wine obtained with 7% alcohol of pH 3.69. In the wineries, the pomace let over, after producing wine is thrown. There is no much information or study about Pomace. As an initiative, the pomace is made into powder in a simple drying method and the powder is analysed for its basic properties. The research is aimed to utilize the pomace powder as a nutrient for aiding the germination of seeds in petri plates. Pomace powder is diluted in 5 different concentrations *viz* 1%, 10%, 25%, 50% and 100% along with control and used for germination study with Moong seeds and Brown Chena. Germination rate and the growth of the sprouts in 7 days in both the seeds in all the treated plates have shown better results than control. With regard to Moong, 25% pomace treated showed very good results compared to others. In Brown chena, 50% pomace treated showed best result of growth. In the pomace treated plates, even the leaves of Moong started to emerge and the length of the sprout is well developed in the pomace treated plates. From the study, it is concluded that the pomace obtained in the wineries containing high level of nutrients that can be made available to the plants and this will result in better growth and development of the crop at less cost.

Keywords: Grapes, fermentation, wine, pomace, analysis and seed germination

1. Introduction

Grape wine is one of the most common alcoholic beverage that has received more research attention due to its industrial commercialization (Bhalla *et al*., 2004) [1]. The grape wine production process involves various steps like crushing of grapes to extract the grape juice, fermentation, storage in bulk and maturating the wine in a wooden or steel cellar, clarification and finally packaging. Though the process seems to be very simple, the quality of the wine depends on the controlled fermentation process and that ensures the product's high quality (Yarrow, 1988) [2]. The typical flavor of grape wine is originated during the processing operations of the raw material, grapes. The terpenes like volatile substances that present in the grapes is responsible for the unique fruity odour of the grape wine. Even non-volatile compounds like tartaric acid and malic acids present in grapes also impact over the flavour of grape wines. The tannins in the grapes provide bitterness and a stringency to grape wine. The tannins are more prominent in red wines as they are the components of grape skins (Vullo *et al.*, 2005) [3]. Although *Saccharomyces cerevisiae* is the principle organism involved in wine preparation, other bacterial groups like filamentous fungi, lactic acid bacteria and acetic acid bacteria, also play a major role in the production of alcoholic grape fruit products. The type and number of organisms present in the wine depends upon the external environmental factors like temperature, stage of maturity, humidity, application of fungicides and so on. Wines were produced by inoculation of *Saccharomyces cerevisiae* culture whereas companion wines were allowed to undergo fermentation by inoculating with indigenous microflora.

Fruit wines are alcoholic beverages that are undistilled and usually made from grapes or fruits like peaches, apricots, plums, etc. Preparation of wine mainly involves fermentation and ageing. Many factors are there that influence the yeast growth rate and fermentation process. The factors like initial sugar concentration of the juice, pH of the must, temperature at which fermentation is carried out and the type of yeast strain used influences wine preparation and its quality. During fermentation, yeast intake sugar present in the grape juice and converts into alcohol, carbon dioxide and heat energy. The time utilised for the fermentation depends on temperature and usually varies from three days to six weeks when the fermentation temperatures are of 65°F to 85°F. Following fermentation, the best ageing time of wine is 6 months to 2 years. The alcohol content of wine could be in the range of 5 to 13% (Anderson, 1970) [4].

Wines are classified based on their colour and sugar content. Based on the color, the wines are classified into three types -

rose wine, red wine and white wine (Zhao, 2005) [5]. Red wine is red in color and prepared from red grape or other fruits that are having red skin. White wine is found to be in the shades of brown or yellow. Rose wine is pink in colour (Anderson, 1970) [4]. Based on the sugar content, the wines are classified as sweet wines and dry wines. Sweet wines are wines that contains 1% or more (up to 14%) residual sugar after fermentation. Dry wines contain less than 1% of residual sugar and are not sweet in taste. The flavour of dry wine is due to the alcohol, enzymes, organic acids, minerals, and esters (Jackson, 2000) [6]. Table wine is a dry wine that contains 9% to 12% alcohol by volume. Table wine is meant to drink with the meal. After-dinner wine includes the popular red and white ports (Gumus and Gumus, 2008) [7]. A Population studies over the health benefits of red and white wine have revealed that wine consumption may reduce the risk of getting heart disease. Another laboratory studies shows that compared to white wines, red wines may help to prevent the cancer due to the presence of polyphenols in red wines (Anderson, 1970) [4].

Recently, there has been an urge in the utilization of the waste materials that are generated in the industries including the wine industry/ grapes processing industries. The generated waste materials in wine industries are organic and are of plant sources and therefore could be an alternative source of natural antioxidants, that are considered to be safe compared to synthetic antioxidants. One of the most abundant waste material generated from wine industry includes grape pomace. It is characterized by high-phenolic contents that are naturally available in grapes and can be utilized as an organic nutrient for sustainable agricultural production (Fontana *et al.*, 2013) [8]. There is a great interest in the extraction of the phenolics present in the grape pomace for obtaining potentially bioactive natural phenolic compounds from a cheap source. The antioxidant flavonoids can also be extracted from the pomace and can be provided as a dietary supplements and thus economically beneficial to the wine industries (Beres *et al*., 2017) [9]. During wine preparation, around 55-65% of the grapes are discarded as waste after extraction of juice. This grapes waste called as pomace includes pulp (57%), skin (25%), stem (10%) and seeds (8%) (Mirabella, 2014) [10]. Following every crush of grapes during the commercial production of grapes juice, around 2T/ha/year with skins, pulp, seeds and stems are left over. The present research is designed to study the feasibility of utilization of tons of unused pomace as a fertilizer for betterment of agriculture.

2. Materials and Methods

The present study was carried out at Biochemistry Division, National Sugar Institute, Kanpur. Chemicals which have been used in study were of high-grade reagents.

2.2.1 Collection of Red Grapes

Red Grapes were collected from the local market in Kalyanpur, Kanpur, Uttar Pradesh.

Fig 1: Red Grapes

2.2.2. Preparation of Grape Juice

Red grapes were washed thoroughly with running tap water and with warm water and then crushed with wooden ladle to collect the juice.

2.2.3. Analysis of grape Juice

The Grape juice obtained was analysed for pH, Brix, Specific Gravity, TRS, RS, Alcohol Percentage, Total Acidity, TDS and TSS parameters following standard procedures.

2.2.4 Preparation of Wine from Grape Juice

A pinch of potassium metabisulphate was added to clarify the Grape juice. The first step in preparation of wine making is to add 2% of active dry yeast (*Saccharomyces cerevisiae*) which utilise the sugar in grape juice to produce ethanol under anaerobic condition. A rubber tube was connected to filter flask with beaker containing water for visualising the. CO_2 bubbles releasing during fermentation process. The whole setup was placed in incubator at 22 to 24 °C for 15 days. The filter flask was shaked twice a day regularly till 15 days. The low temperature slow fermentation favours the retention of volatile compounds. After 15 days, the wine was analysed for Brix, pH, Acidity, TRS, RS and Specific Gravity.

2.2.5. Filtration of Wine

After 15 days of fermentation, filter the wine and the solid matter collected is referred as Pomace. The filtrate obtained was again kept for incubation for 15 days. The pomace collected was stored in refrigerator for further study. After 30 days, the wine was analysed for Brix, pH, Acidity, TRS, RS and Specific Gravity. The particles suspended in fermentation broth are separated by centrifugation and

collected the supernatant using ice bath. The wine was bottled after analysing alcohol and total Acidity.

2.2.6 Analysis of Pomace Powder
Pomace collected (Filtered) during Grape wine production were dried in oven at 70 °C for 48 hours and cooled down. The dried pomace is made into fine powder and used for further study. Took 1gm of pomace and dissolved in 100 ml of tap water to makes into solution and was analysed for pH, TDS, COD and Moisture parameters using standard procedures.

2.2.7 Germination of Seed Using Pomace
Germination study was carried out with the pomace powder using petri plates. The experiment was formulated in duplicates with six treatments *viz* 1%, 10%, 25%, 50% and 100% Pomace solution and a control for comparison. The normal tap water is used as control. For germination study in petri plates, two seeds namely Brown chena and Moong beans are used. Uniform seeds are selected, washed and then used. In pre-sterilised petri dishes, whatman no.1 filter paper is placed and wetted with respective pomace solutions and one set with a control – water. Ten seeds of each variety are sown over each treated filter paper (i.e) wetted with different concentration of pomace solution and observed for 7 days. The number of seeds germinated after 7 days was counted for the study of percentage of germination.

3. Results and Discussions
Grapes [*Vitis* spp.] are cultivated throughout the world and therfore different species and or varieties are developed (Sargolzaei *et al*., 2021) [11]. Grapes of Vitis species are of three types: *Vitaceae V. vinifera*: European grapevines, *V. labrusca*: American grapevines and *V. rotundifolia*: Muscadine grapevines (Rehman *et al*., 2018) [12]. *V. vinifera*, grapevine is the most widely planted grape species that are used for the production of wine, juice and raisin (Sargolzaei *et al.*, 2021) [11]. Grapevine fruit is a type of berry that could be red, white or purple color. The major pigment that is present in Grapevine include Anthocyanin and their concentration vary with different varieties. Major anthocyanins distributed in grapes include derivatives of cyanidin, delphinidin, petunidin, malvidin and peonidin and all of these compounds are biosynthesized through flavanoid pathway. The skin color of the grapes is influenced by the Anthocyanins thereby influences the juice and wine color and color stability through storage (Ju *et al*., 2021) [13]. The color difference in the natural pigments differ in content according to the factors like genetic, environmental and agronomical conditions (Quina & Bastos, 2018) [14]. Very recently it is shown that, the mutation of red grapes resulted in white ones (Ferreira *et al*., 2019) [15]. The presence of high sugar, pectin, aromatic compounds and high levels of acids, especially tartaric acid in grapes make it amenable to many different end uses.

3.1 Analysis of Grape Juice
The Grape juice was analysed for following physical parameters like P^H, Brix, Specific gravity, TRS, RS, Alcohol percentage, Total Acidity, TDS and TSS. The results of the analysis are given below in the Table 1.

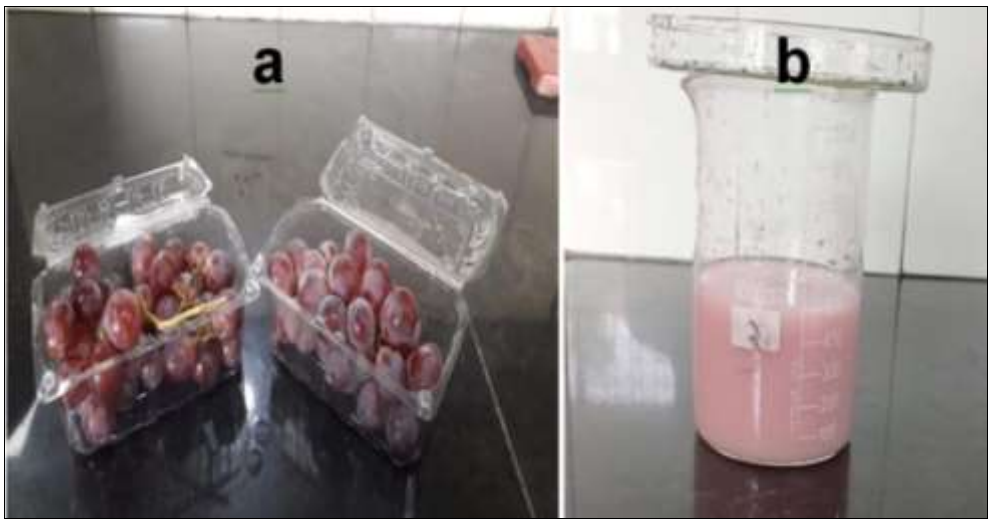

Fig 2: (a) Red Grapes (b) Red Grape juice

Table 1: General characteristics of Grape juice

S. No	Properties	Experimental value
1.	P^H	4.72
2.	BRIX °	20
3.	SPECIFIC GRAVITY	1.088
4.	TRS %	5.23
5.	RS %	4.25
6.	ALCOHOL %	0.05
7.	TOTAL ACIDITY %	0.06
8.	TDS in ppm	0.86
9.	TSS gm /ml	1.8

3.2 Fermentation of Grape Juice

After analysing the red grape juice for various above said parameters, yeast is added and kept for fermentation at 20° C as shown in the figure 1.

Fig 3: Grape Juice for Fermentation

3.3 Analysis of Grape wine

After 15 days, the wine was analysed for the following parameters P^H, Brix, Specific gravity, TRS, RS and Total Acidity and the results obtained are given in the Table 2.

Table 2: Analysis of wine after 15 days

S. No	Parameters	Values
1.	P^H	3.69
2.	Brix °	20
3.	Specific Gravity	1.044
4.	TRS %	5.23
5.	RS %	4.25
6.	Total Acidity %	1.2
7.	Alcohol %	5

3.4 Analysis of wine after 30 days

After 30 days, the wine was analysed for the following parameters P^H, Brix, Alcohol percentage and Total Acidity and the results obtained are given in the Table 3.

Table 3: Analysis of wine after 30 days

S. No.	Parameters	Values
1.	PH	3.69
2.	Brix °	1.043
3.	Total Acidity %	1.2
4.	Alcohol %	7

3.5 Analysis of Grape Pomace

Grape pomace which is removed from wine through filtration is dried in oven and made into fine powder as shown in the Fig 2. The dried pomace powder is analysed and the results obtained is reported in the Table 4.

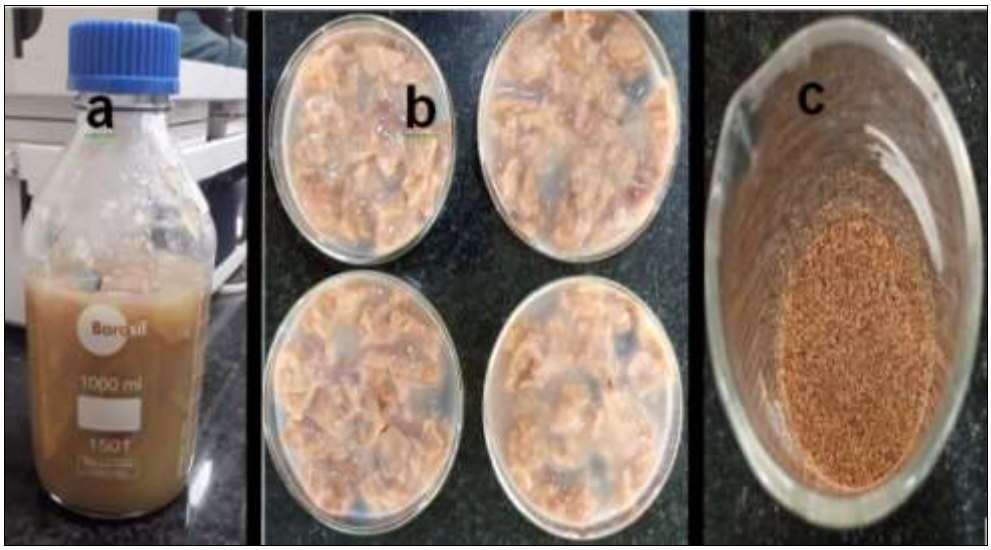

Fig 4: (a) Pomace (b) Pomace kept in oven for drying (c) Pomace powder

Table 4: Analysis of Grape Pomace

S. No.	Parameters	Values
1.	P^H	4.84
2.	TDS	0.77ppt
3.	COD	48000 mg/l
4.	MOISTURE	95.27%

3.5 Germination of Seed using Pomace

The germination study results of 2 different seedlings – Moong and Chena Dal with different diluted proportions of pomace powder after 7 days of sowing was depicted in the Table 5. The experiment was carried out in duplicates and in each petriplate 10 seeds were kept and observed for germination. Initially 10 ml of treated water was added and thereafter everyday 1 ml of pomace diluted water is added in all the treatments till the end of the 7th day of sowing. Then the number of seeds germinated in each set is averaged and noted down. The same is given in the Fig 3.

Table 5: Germination of Seeds in Petri Plates

Treatment	Number of seeds germinated after 7 days/out of 10 seeds sown			
	Moong		Brown chena	
	No:	%	No:	%
C	7	70	8	80
1%	8	80	8	80
10%	9	90	9	90
25%	10	100	9	90
50%	10	100	8	80
100%	10	100	8	80

Fig 5: Moong and Brown Chena seeds plated in petri dishes

Fig 6: Germination of seeds after 7 days

The results observed reveals that both Moong and Brown Chena seeds showed better germination and growth in all the pomace treated plates compared to Control. In case of Moong, 25% pomace treated showed very good results compared to others. In Brown chena, 50% pomace treated showed best result of growth. In the pomace treated plates, even the leaves of Moong started to emerge and the length of the sprout is well developed in the pomace treated plates.

Conclusion

In the present research, the wine is prepared at laboratory conditions using Red grapes. The produced wine is analysed for its physical properties for every 15days interval *viz* P^H, TRS, RS, Specific Gravity, Brix, TDS and TSS. The final wine obtained with 7% alcohol of P^H 3.69. In the wineries, the pomace let over, after producing wine is thrown. There is no much information or study about Pomace. As an initiative, the pomace is made into powder in a simple drying method and the powder is analysed for its basic properties. The research is aimed to utilize the pomace powder as a nutrient for aiding the germination of seeds in petriplates. Pomace powder is diluted in 5 different concentrations *viz* 1%, 10%, 25%, 50% and 100% along with control and used for germination study with Moong seeds and Brown Chena. Germination rate and the growth of the sprouts in 7 days in both the seeds in all the treated plates have shown better results than control. With regard to Moong, 25% pomace treated showed very good results compared to others. In Brown chena, 50% pomace treated showed best result of growth. In the pomace treated plates, even the leaves of Moong started to emerge and the length of the sprout is well developed in the pomace treated plates. From the study, it is concluded that the pomace obtained in the wineries containing high level of nutrients that can be made available to the plants and this will result in better growth and development of the crop at less cost.

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Author Contributions

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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