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Development and Evaluation of Autoclavable Lab Scale Fermenter

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ABSTRACT

A lab scale autoclavable fermenter was developed with working capacity of 3L out of the total volume of 4 L for the production of red wine from local variety of grapes and evaluated in the developed fermenter by changing the temperature and speed of the agitator to study the favorable conditions for fermentation. The temperature ranges of 28, 30 and 32°C and the agitator speed of 0, 100 and 200 rpm were set to evaluate the developed fermenter in terms of alcohol recovery in per cent. Fermentation initiated with brewer's yeast of 2% at the fermentation temperature of 28°C resulted in the maximum yield of 12.4 per cent alcohol from the grape must in the developed fermenter. Fermentation at 30 and 32°C were yielded comparatively low, lost some colour and stuck fermentation results. It was also produced raisin like falvour instead of wine fruity flavor. Variations in the agitator speed at the fermentation temperature of 28°C did not show any significant effect on the alcohol recovery. Hence, it was decided to conduct fermentation at 28°C without any agitation in the fermenter.

Keywords *Lab scale autoclavable fermenter, grape wine, alcohol recovery, temperature, (RPM) roatation per minute.*

Grapes (*Vitis* spp.) are economically important fruit species in the world primarily for wine production. In India, total area of grapes cultivation was 88,000 ha with annual production of 24, 54,000 metric tons during the year 2014-15 (Kumar *et al*., 2017). The conversion of grape juice to wine is a biotechnological process. Wine making begins with the collection and crushing of grapes. There are two types of wine namely white wine and red wine. The sugar fermentation phase is dominated primarily by *Sac-charomyces cerevisiae*, a yeast that has been extensively studied in wine production. It was the first eukaryotic organism to have its genome sequenced (Goffeau *et al*., 1996) and widely used in wine fermentation industrially. There are two kinds of wine productions such as red and white wine.

Tesfaye *et al*. (2000) used a laboratory scale fermenter comprised of a cylindrical concave bottom glass culture vessel of 5 litre capacity with a height-to-diameter ratio of 2:1, an air supply system with air filters and inlet pipe with sparger ring, a refrigeration system with cold water to prevent loss of volatile components, electrical heater jacket 230V and cooling system of the vessel with simple water bath, stirrer with 6-bladed disc impellers, Pt-100 pH-electrode, pO2-electrode, sensor for temperature measurement Pt-100, measurement and control system micro-DCU 300, stirrer speed control MCU-200 and dosing pump-300. Ferreira *et al.* (2010) has stated that fermentation efficiency is also directly related to the stress resistance, i.e. the ability of yeasts to respond efficiently to a changing environment and unfavorable growth conditions (Bauer and Pretorius 2000).

MATERIALS AND METHODS

Development of fermenter

Fermenter was developed for the purpose of conducting preliminary studies on grape wine production under the controlled conditions. A 4 L capacity autoclavable glass fermenter was developed by assembling the following accessories:

1. Body - Borosilicate glass
2. Top - SS 316L
3. Seal - Silicon lip seal
4. Turbine - 3 No's
5. Total volume - 4.3 L
6. Agitator - 180 W

The borosilicate container (make: Borg) of 4 L capacity was fitted with top lid made up of stainless steel 316 L. The lid was attached with port facilities such as sample port, CO_2 outlet, cooling water in and out, pH probe port and thermometer port.

pH meter :- 0 to 14

Thermometer :- 0-100°C

Agitator :- 100 to 1000 rpm

Evaluation of developed fermenter

Under the anaerobic condition, 3 L volume of grape must fermentation was carried out and evaluated by changing the temperature and rpm of impellers provided in the fermenter. The temperature of 28, 30 and 32°C were maintained in different period of time interval. In that later cases, slight change in colour due to high temperature and stuck fermentation results. Hence, it found that 28°C was the optimum temperature for red wine making in the small scale fermenter. Revolution of agitator was varied viz., 0, 100, 200 rpm at 28°C to study the impact of agitation on the fermentation of grape must. At the end of fermentation sediment settled at the bottom of fermenter were removed and clear wine was collected and the amount of alcohol recovery was studied.

Flowchart of steps in the winemaking is presented in Fig-1

Selection of wine grapes (Mature and undamaged grapes)

↓

Grape berries washed and crushed (Manually under hygienic conditions)

↓

Grape skins and seeds were remaining in juice for extraction of colour and phenolic compounds (kept overnight for aerobic fermentation)

↓

Adjust TSS 22°B by adding cane sugar

↓

Addition of Culture (2%)

↓

Fermenter at 28°C

↓

Filtration using filters

↓

Racking and Siphoning

↓

Clarification(Filter / muslin cloth)

↓

Deacidification by slight heating

↓

Bottling and storage

Fig. 1. Methodology

(According BIS Standards- https://archive.org/details/gov.in.is.7058.2005)

Plate 1. Wine making
a) Grapes, b) Blanching, c) grape must, d) fermeneter, e) Brewer's Yeast, f) wine

Table 1. Evaluation of developed fermenter

Independent variables	Dependent variables	
Temperature(ºC)	**Alcohol recovery (%)**	**Mean**
28	12.4	12.4±0.07[c]
30	11.5	11.5±0.09[b]
32	11	11±0.06[a]
F-value		**90.600****
RPM	**Alcohol recovery (%)**	**Mean**
0	12	12±0.04[a]
100	12	12±0.06[a]
200	11.9	11.9±0.04[a]
F-value		**1.412[NS]**

RESULTS AND DISCUSSION

Development of Fermenter

A lab scale fermenter was developed with working capacity of 3 L out of 4 L total volume. The fermenter was provided with stainless steel with provision for ports to accommodate agitator, pH probe, cold water inlet and outlet and thermometer. The rated power for agitator was 180 W with provision to change the speed of impeller. The main purpose of the fermenter was to study the suitable parameters that favour the fermentation positively.

Evaluation of developed fermenter

Studies were conducted in the developed fermenter by changing the temperature and speed of the agitator to study the favorable conditions for fermentation. The temperatures varied at 28, 30 and 32 °C and the agitator rpm was set 0, 100 and 200. It was found from table 4.1 that temperature changes had significant effect on the alcohol recovery at 1 per cent level of interval. The highest mean alcohol recovery of 12.4 per cent was noticed at the fermentation temperature of 28°C whereas higher temperatures showed low alcohol recovery. Hence, it was decided to conduct the study on fermentation after pretreatments at 28°C. Considering the intervention of agitator speed on the fermentation, the rpm levels were set 0, 100 and 200 in the developed fermenter at the fermentation temperature of 28 °C. It was again noticed from the table 4.1 that variation in the agitator speed did not have any significant effect on the alcohol recovery. Hence, it was decided not to give any agitation during fermentation after pretreatments of the grape must at 28°C.

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